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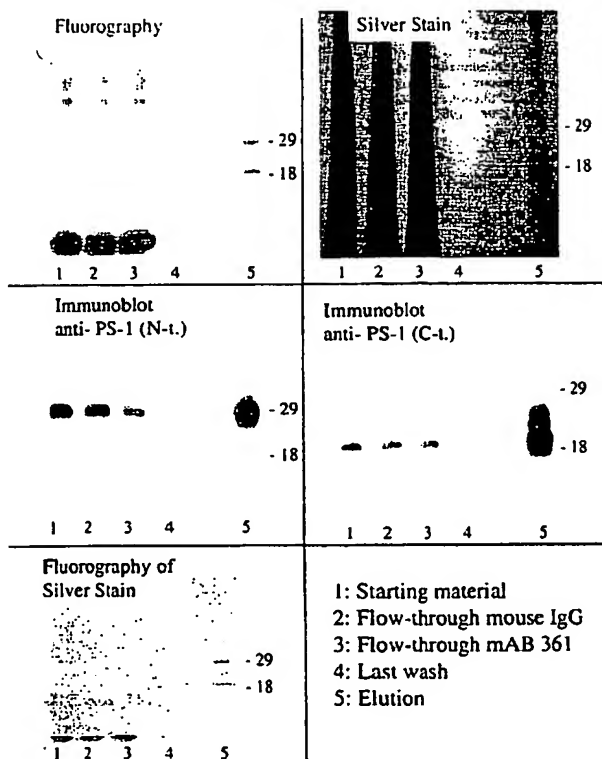
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(54) Title: **USE OF SMALL MOLECULE RADIOLIGANDS TO DISCOVER INHIBITORS OF AMYLOID-BETA PEPTIDE PRODUCTION AND FOR DIAGNOSTIC IMAGING**



(57) Abstract: This invention relates to a method of using radiolabelled and/or radiopharmaceutical small molecule inhibitors of beta-amyloid peptide production for the diagnosis of neurological and other disorders involving APP processing and beta-amyloid production. Furthermore, radiolabelled small molecule inhibitors identified by the methods of the present invention would be useful in the diagnosis of neurological disorders, such as Alzheimer's disease, which involve elevated levels of A β peptides.



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TITLE

USE OF SMALL MOLECULE RADIOLIGANDS TO DISCOVER INHIBITORS
OF AMYLOID-BETA PEPTIDE PRODUCTION AND FOR DIAGNOSTIC
IMAGING

5

FIELD OF THE INVENTION

This invention relates to a method of using
radiolabelled and/or radiopharmaceutical small molecule
inhibitors of beta-amyloid peptide production for the
10 diagnosis of neurological and other disorders involving APP
processing and beta-amyloid production. Furthermore,
radiolabelled small molecule inhibitors identified by the
methods of the present invention would be useful in the
diagnosis of neurological disorders, such as Alzheimer's
15 disease, which involve elevated levels of A β peptides.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is a degenerative brain
disorder characterized clinically by progressive loss of
20 memory, temporal and local orientation, cognition,
reasoning, judgment and emotional stability. AD is a
common cause of progressive dementia in humans and is one
of the major causes of death in the United States. AD has
been observed in all races and ethnic groups worldwide, and
25 is a major present and future health problem. No treatment
that effectively prevents AD or reverses the clinical
symptoms and underlying pathophysiology is currently
available (for review, Dennis J. Selkoe; Cell Biology of
the amyloid (beta)-protein precursor and the mechanism of
30 Alzheimer's disease, Annu Rev Cell Biol, 1994, 10: 373-
403).

Histopathological examination of brain tissue derived
upon autopsy or from neurosurgical specimens in effected
individuals revealed the occurrence of amyloid plaques and
35 neurofibrillar tangles in the cerebral cortex of such
patients. Similar alterations were observed in patients
with Trisomy 21 (Down's syndrome), and hereditary cerebral
hemorrhage with amyloidosis of the Dutch-type.

Neurofibrillar tangles are nonmembrane-bound bundles of abnormal proteinaceous filaments and biochemical and immunochemical studies led to the conclusion that their principle protein subunit is an altered phosphorylated form of the tau protein (reviewed in Selkoe, 1994).

Biochemical and immunological studies revealed that the dominant proteinaceous component of the amyloid plaque is an approximately 4.2 kilodalton (kD) protein of about 39 to 43 amino acids. This protein was designated A β , β -amyloid peptide, and sometimes β /A4; referred to herein as A β . In addition to deposition of A β in amyloid plaques, A β is also found in the walls of meningeal and parenchymal arterioles, small arteries, capillaries, and sometimes, venules. A β was first purified, and a partial amino acid reported, in 1984 (Glenner and Wong, Biochem. Biophys. Res. Commun. 120: 885-890). The isolation and sequence data for the first 28 amino acids are described in U.S. Pat. No 4,666,829.

Compelling evidence accumulated during the last decade revealed that A β is an internal polypeptide derived from a type 1 integral membrane protein, termed β amyloid precursor protein (APP). β APP is normally produced by many cells both in vivo and in cultured cells, derived from various animals and humans. A β is derived from cleavage of β APP by as yet unknown enzyme (protease) system(s), collectively termed secretases.

The existence of at least four proteolytic activities has been postulated. They include β secretase(s), generating the N-terminus of A β , α secretase(s) cleaving around the 16/17 peptide bond in A β , and γ secretases, generating C-terminal A β fragments ending at position 38, 39, 40, 42, and 43 or generating C-terminal extended precursors which are subsequently truncated to the above polypeptides.

The gene encoding a human aspartic protease that cleaves the β -secretase site of β -amyloid precursor protein has recently been isolated; this gene and encoded protein is designated as BACE (Vassar et al., Science (1999) 286:

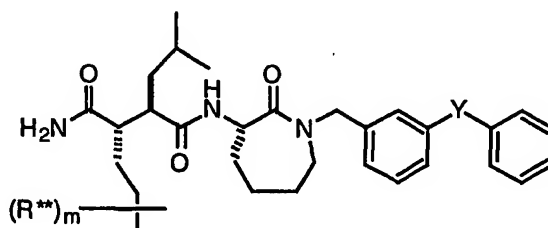
735-741) or as memapsin-2 (Lin et al., PNAS (2000) 97: 1456-1460) and is designated herein as "BACE/memapsin-2".

Several lines of evidence suggest that abnormal accumulation of A β plays a key role in the pathogenesis of AD. Firstly, A β is the major protein found in amyloid plaques. Secondly, A β is neurotoxic and may be causally related to neuronal death observed in AD patients. Thirdly, missense DNA mutations at position 717 in the 770 isoform of β APP can be found in effected members but not unaffected members of several families with a genetically determined (familiar) form of AD. In addition, several other β APP mutations have been described in familial forms of AD. Fourthly, similar neuropathological changes have been observed in transgenic animals overexpressing mutant forms of human β APP. Fifthly, individuals with Down's syndrome have an increased gene dosage of β APP and develop early-onset AD. Taken together, these observations strongly suggest that A β depositions may be causally related to the AD.

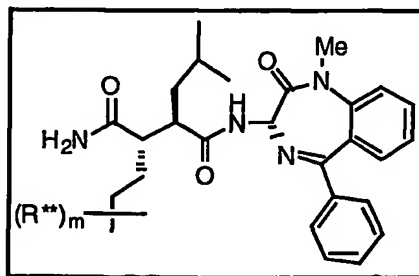
It is hypothesized that inhibiting the production of A β will prevent and reduce neurological degeneration, by controlling the formation of amyloid plaques, reducing neurotoxicity and, generally, mediating the pathology associated with A β production. One method of treatment methods would therefore be based on drugs that inhibit the formation of A β in vivo.

Methods of treatment could target the formation of A β through the enzymes involved in the proteolytic processing of β amyloid precursor protein. Compounds that inhibit β or γ secretase activity, either directly or indirectly, could control the production of A β . Advantageously, compounds that specifically target γ secretases, could control the production of A β . Such inhibition of β or γ secretases could thereby reduce production of A β , which, thereby, could reduce or prevent the neurological disorders associated with A β protein.

It is believed that several macromolecules, some of which have proteolytic activity, are involved in the processing of amyloid precursor protein (APP). This processing leads to several products including the β -amyloid peptides ($A\beta$) believed etiologically important in Alzheimers Disease. We have discovered novel tagged compounds, functional in themselves as $A\beta$ inhibitors, for use in identifying a site or sites on one or more macromolecules critical to the processing of β APP and the production of $A\beta$. We have discovered novel tagged compounds which inhibit the proteolytic activity leading to production of $A\beta$ by interacting with one or more macromolecules critical to the processing of APP and the production of $A\beta$. We have also discovered a site of action of these tagged compounds using radioisotope tagged derivatives of a compound of Formula (I). Three examples of tagged compounds include (I-7T), (I-11T), and (I-43T):



(I-#)

(I-7) : $R^{**} = {}^1H$; $Y = -O-$;(I-7T) : $R^{**} = {}^3H$; $Y = -O-$; and(I-11) : $R^{**} = {}^1H$; $Y = -C(=O)-$;(I-11T) : $R^{**} = {}^3H$; $Y = -C(=O)-$; and

(I-#)

(I-43) : R** = ^1H ;

(I-43T): R** = ^3H .

5 The concentration of Compound (I-7) leading to half-maximal inhibition (IC_{50}) of proteolytic activity leading to $\text{A}\beta$ production in HEK_{293} cells expressing APP 695 wt is similar to the concentration leading to half-maximal inhibition (IC_{50}) of Compound (I-7T) binding to membranes derived from the same cell line. The correlation holds for
10 compounds (I-11T) and (I-43T). Also using a compound of Formula (I), we have discovered a macromolecule containing a binding site of action for compounds of Formula (I) critical to the processing of APP and the production of $\text{A}\beta$.

Furthermore, we have discovered through competitive
15 binding studies that there is a good correlation between the ability of a series of compounds to inhibit the proteolytic activity leading to production of $\text{A}\beta$ and to inhibit the binding of Compound (I-7T), (I-11T), or (I-43T) to said membranes. Thus, the binding of Compound (I-7T),
20 (I-11T), or (I-43T) to relevant tissues and cell lines, membranes derived from relevant tissues and cell lines, as well as isolated macromolecules and complexes of isolated macromolecules, is useful in the identification of inhibitors of $\text{A}\beta$ production through competitive binding
25 assays. Furthermore, such competitive binding assays are useful in identification of inhibitors of proteolytic activity leading to $\text{A}\beta$ production for the treatment of Alzheimer's disease. Furthermore, such competitive binding assays are useful in identification of inhibitors of
30 proteolytic activity leading to $\text{A}\beta$ production for the treatment of neurological disorders and other disorders involving $\text{A}\beta$, APP, and/or $\text{A}\beta$ /APP associated macromolecules, and other macromolecules associated with the site of Compound (I-7T), (I-11T), or (I-43T) binding.

35

SUMMARY OF THE INVENTION

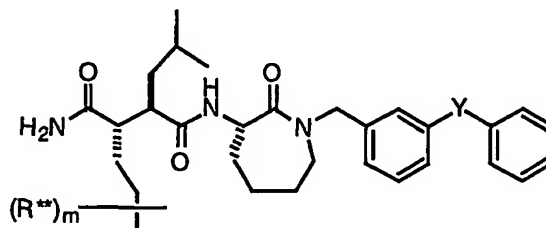
One object of the present invention is to provide a method for diagnosing a neurological disease involving APP

processing and/or the production of beta-amyloid production within a mammalian body comprising: a) administering a diagnostically effective amount of a radiopharmaceutical inhibitor of APP processing and/or the production of beta-amyloid production; and b) imaging the area of the patient wherein the disease is located.

It is another object of the present invention to provide a method for diagnosing a neurological disease involving APP processing and/or the production of beta-amyloid production within a mammalian body comprising: a) administering a diagnostically effective amount of an ultrasound contrast agent composition inhibitor of APP processing and/or the production of beta-amyloid production; and b) imaging the area of the patient wherein the disease is located.

It is another object of the present invention to provide radiolabeled inhibitors of APP processing and/or the production of beta-amyloid production for use in methods of in vivo diagnostic imaging in the diagnosis of diseases involving APP processing and/or the production of beta-amyloid production. Also provided in the present invention are methods of in vivo diagnostic imaging comprising administering to a subject a diagnostically effective amount of a radiolabeled inhibitor of APP processing and/or the production of beta-amyloid production

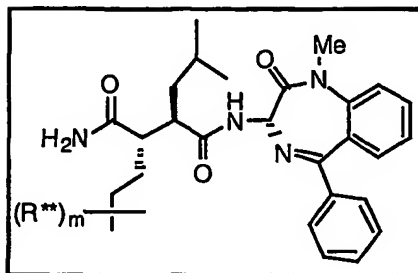
These and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that compounds of Formula (I):



(I-#)

(I-7) : $R^{**} = {}^1H$; $Y = -O-$;

(I-7T): $R^{**} = {}^3H$; $Y = -O-$; and
 (I-11): $R^{**} = {}^1H$; $Y = -C(=O)-$;
 (I-11T): $R^{**} = {}^3H$; $Y = -C(=O)-$; and



(I-#)

(I-43) : $R^{**} = {}^1H$;
 (I-43T): $R^{**} = {}^3H$;

- 10 bind specifically to a binding site on a macromolecule or a complex of macromolecules involved in APP processing to produce reduction of A β peptide production. For example, the concentration of Compound (I-7) leading to half-maximal inhibition (IC_{50}) of A β production in HEK₂₉₃ cells expressing
- 15 APP 695 wt is similar to the concentration leading to half-maximal inhibition (IC_{50}) of Compound (I-7T) binding to membranes derived from the same cell line.

FIG. 1 illustrates the correlation between results of the

20 Radioligand Competition Binding Assay and the cross-linking assay of Example 103.

FIG. 2 illustrates a fluorography of a 12% SDS-PAGE after immunoprecipitation of specifically cross-linked polypeptides by presenilin-1 antibodies.

25 FIG. 3 illustrates isolation of cross-linked polypeptides by presenilin 1 affinity chromatography.

FIG. 4 illustrates a fluorography of a 12% SDS-PAGE after immunoprecipitation of specifically cross-linked polypeptides by presenilin-2 antibodies.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[1] Thus, in a first embodiment, the present invention provides a method of screening for inhibitors of beta-

5 amyloid production comprising,

- 1) contacting a potential inhibitor of beta-amyloid production and a tagged inhibitor of beta-amyloid production with at least one macromolecule involved in the processing of APP and/or the production of beta-amyloid peptide, said macromolecule containing a
10 binding site specific for said tagged inhibitor of beta-amyloid production;
- 2) separating the tagged inhibitor of beta-amyloid production bound to said macromolecule from the tagged
15 inhibitor of beta-amyloid production free from said macromolecule; and
- 3) determining an inhibitory concentration of the potential inhibitor of beta-amyloid production from the concentration of tagged inhibitor of beta-amyloid
20 production bound to said macromolecule.

[1a] The present invention provides the foregoing method wherein the macromolecule is selected from:

- (1) presenilin-1;
- 25 (2) presenilin-2;
- (3) β secretase;
- (4) α secretase;
- (5) γ secretase; or
- (6) BACE/memapsin 2.

30

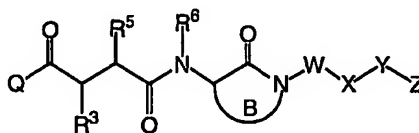
[2] In a more preferred embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a radiolabeled inhibitor of beta-amyloid production, a fluorescence labeled inhibitor
35 of beta-amyloid production or a biotin labeled inhibitor of beta-amyloid production.

[3] In a more preferred embodiment the tagged inhibitor of beta-amyloid production comprises a radiolabeled inhibitor of beta-amyloid production.

5 [4] In an even more preferred embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a tritium or iodine radiolabeled inhibitor of beta-amyloid production.

10 [5] In an even more preferred embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a tritium labeled inhibitor of beta-amyloid production.

15 [6] In an even more preferred embodiment the present invention, provides a method wherein the tagged inhibitor of beta-amyloid production comprises a compound of the Formula (I):



(I)

wherein:

at least one atom of the compound of the Formula (I) is radiolabeled;

Q is -NR¹R²;

R¹, at each occurrence, is independently selected from:

H;

C₁-C₆ alkyl substituted with 0-3 R^{1a};

C₃-C₁₀ carbocycle substituted with 0-3 R^{1b};

C₆-C₁₀ aryl substituted with 0-3 R^{1b}; and

5 to 10 membered heterocycle substituted with 0-3 R^{1b};

- R^{1a}, at each occurrence, is independently selected from H,
C₁-C₆ alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶,
phenyl, CF₃;
C₃-C₁₀ carbocycle substituted with 0-3 R^{1b};
5 C₆-C₁₀ aryl substituted with 0-3 R^{1b}; and
5 to 10 membered heterocycle substituted with 0-3 R^{1b};
- R^{1b}, at each occurrence, is independently selected from H,
OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂,
10 NR¹⁵R¹⁶, or CF₃;
- R² is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₆
alkoxy, C₃-C₁₀ carbocycle, C₆-C₁₀ aryl and 5 to 10
membered heterocycle;
15
- R³ is C₁-C₆ alkyl substituted with 0-1 R⁴;
- R⁴ is H, OH, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆
alkynyl, C₃-C₁₀ carbocycle, C₆-C₁₀ aryl, or 5 to 10
20 membered heterocycle;
- R⁵ is H, OR¹⁴;
C₁-C₆ alkyl substituted with 0-3 R^{5b};
C₁-C₆ alkoxy substituted with 0-3 R^{5b};
25 C₂-C₆ alkenyl substituted with 0-3 R^{5b};
C₂-C₆ alkynyl substituted with 0-3 R^{5b};
C₃-C₁₀ carbocycle substituted with 0-3 R^{5c};
C₆-C₁₀ aryl substituted with 0-3 R^{5c}; or
5 to 10 membered heterocycle substituted with 0-3 R^{5c};
30
- R^{5b}, at each occurrence, is independently selected from:
H, C₁-C₆ alkyl, CF₃, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂,
NR¹⁵R¹⁶;
C₃-C₁₀ carbocycle substituted with 0-3 R^{5c};
35 C₆-C₁₀ aryl substituted with 0-3 R^{5c}; or
5 to 10 membered heterocycle substituted with 0-3 R^{5c};

R^{5c}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;

5 R⁶ is H;

C₁-C₆ alkyl substituted with 0-3 R^{6a};

C₃-C₁₀ carbocycle substituted with 0-3 R^{6b}; or

C₆-C₁₀ aryl substituted with 0-3 R^{6b};

10 R^{6a}, at each occurrence, is independently selected from H, C₁-C₆ alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶, phenyl or CF₃;

15 R^{6b}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;

W is -(CR⁸R^{8a})_p-;

20 p is 0 to 4;

R⁸ and R^{8a}, at each occurrence, are independently selected from H, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₂-C₄ alkynyl and C₃-C₈ cycloalkyl;

25

X is a bond;

C₆-C₁₀ aryl substituted with 0-3 R^{Xb};

C₃-C₁₀ carbocycle substituted with 0-3 R^{Xb}; or

5 to 10 membered heterocycle substituted with 0-3 R^{Xb};

30

R^{Xb}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;

35 Y is a bond or -(CR⁹R^{9a})_t-V-(CR⁹R^{9a})_u-;

t is 0 to 3;

u is 0 to 3;

R⁹ and R^{9a}, at each occurrence, are independently selected from H, C₁-C₆ alkyl or C₃-C₈ cycloalkyl;

5

V is a bond, -C(=O)-, -O-, -S-, -S(=O)-, -S(=O)₂-, -N(R¹⁹)-, -C(=O)NR^{19b}-, -NR^{19b}C(=O)-, -NR^{19b}S(=O)₂-, -S(=O)₂NR^{19b}-, -NR^{19b}S(=O)-, -S(=O)NR^{19b}-, -C(=O)O-, or -OC(=O)-;

10 Z is H;

C₁-C₈ alkyl substituted with 0-2 R¹²;

C₂-C₄ alkenyl substituted with 0-2 R¹²;

C₂-C₄ alkynyl substituted with 0-2 R¹²;

C₆-C₁₀ aryl substituted with 0-4 R^{12b};

15 C₃-C₁₀ carbocycle substituted with 0-4 R^{12b}; or

5 to 10 membered heterocycle substituted with 0-3 R^{12b};

R¹² is C₆-C₁₀ aryl substituted with 0-4 R^{12b};

C₃-C₁₀ carbocycle substituted with 0-4 R^{12b}; or

20 5 to 10 membered heterocycle substituted with 0-3 R^{12b};

R^{12b}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;

25

B is a 5 to 10 membered lactam, wherein the lactam is saturated, partially saturated or unsaturated; wherein each additional lactam carbon is substituted with 0-2 R¹¹; and, optionally, the lactam contains a heteroatom selected from -O-, -S-, -S(=O)-, -S(=O)₂-, -N= and -N(R¹⁰)-;

30

R¹⁰ is H, C(=O)R¹⁷, C(=O)OR¹⁷, C(=O)NR¹⁸R¹⁹, S(=O)₂NR¹⁸R¹⁹, S(=O)₂R¹⁷;

35 C₁-C₆ alkyl optionally substituted with R^{10a};

C₆-C₁₀ aryl substituted with 0-4 R^{10b};

C₃-C₁₀ carbocycle substituted with 0-3 R^{10b}; or

5 to 10 membered heterocycle optionally substituted
with 0-3 R^{10b};

5 R^{10a}, at each occurrence, is independently selected from H,
C₁-C₆ alkyl, C₃-C₆ cycloalkyl, OR¹⁴, Cl, F, Br, I, =O,
CN, NO₂, NR¹⁵R¹⁶, phenyl or CF₃;

10 R^{10b}, at each occurrence, is independently selected from H,
OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂,
NR¹⁵R¹⁶, or CF₃;

15 R¹¹ is C₁-C₄ alkoxy, Cl, F, Br, I, =O, CN, NO₂, NR¹⁸R¹⁹,
C(=O)R¹⁷, C(=O)OR¹⁷, C(=O)NR¹⁸R¹⁹, S(=O)₂NR¹⁸R¹⁹, CF₃;
C₁-C₆ alkyl optionally substituted with R^{11a};
C₆-C₁₀ aryl substituted with 0-3 R^{11b};
C₃-C₁₀ carbocycle substituted with 0-3 R^{11b}; or
5 to 10 membered heterocycle substituted with 0-3 R^{11b};

20 alternatively, two R¹¹ substituents on the same carbon
atoms may be combined to form a C₃-C₆ carbocycle;

25 alternatively, two R¹¹ substituents on adjacent carbon
atoms may be combined to form a C₃-C₆ carbocycle or a
benzo fused radical, wherein said benzo fused radical
is substituted with 0-3 R¹³;

30 R^{11a}, at each occurrence, is independently selected from H,
C₁-C₆ alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶,
phenyl or CF₃;

R^{11b}, at each occurrence, is independently selected from H,
OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂,
NR¹⁵R¹⁶, or CF₃;

35 R¹³, at each occurrence, is independently selected from H,
OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂,
NR¹⁵R¹⁶, or CF₃;

R¹⁴ is H, phenyl, benzyl, C₁-C₆ alkyl, or C₂-C₆ alkoxyalkyl;

R¹⁵, at each occurrence, is independently selected from H,
C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl)
5 and -S(=O)₂-(C₁-C₆ alkyl);

R¹⁶, at each occurrence, is independently selected from H,
OH, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆
alkyl) and -S(=O)₂-(C₁-C₆ alkyl);

10

R¹⁷ is H, phenyl, benzyl, C₁-C₆ alkyl, or C₂-C₆ alkoxyalkyl;

R¹⁸, at each occurrence, is independently selected from H,
C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl)
15 and -S(=O)₂-(C₁-C₆ alkyl); and

R¹⁹, at each occurrence, is independently selected from H,
OH, C₁-C₆ alkyl, phenyl, benzyl, phenethyl, -C(=O)-(C₁-
C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);

20

R^{19b} is H, C₁-C₆ alkyl, C₃-C₈ cycloalkyl, phenyl, benzyl or
phenethyl; and

R²⁰ is H or C₁-C₆ alkyl.

25

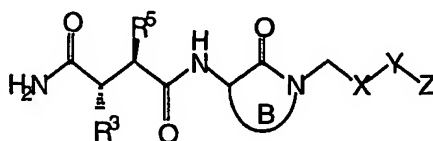
[] In an even further more preferred embodiment the
present invention, provides a method wherein Q of a
compound of Formula (I) is -NH₂.

30 [7] In an even further more preferred embodiment the
present invention, provides a method wherein R³ of a
compound of Formula (I) is C₃-C₆ alkyl.

[8] In an even further more preferred embodiment the
35 present invention, provides a method wherein R³ of a
compound of Formula (I) is C₃-C₆ alkyl substituted with
about 1 to about 4 ³H;

[] In an even further more preferred embodiment the present invention, provides a method wherein Q is $-\text{NH}_2$, and R^3 is $\text{C}_3\text{-C}_6$ alkyl substituted with about 1 to about 4 ^3H .

- 5 [9] In an even further more preferred embodiment the present invention, provides a method wherein the tagged inhibitor of beta-amyloid production comprises a compound of the Formula (II):



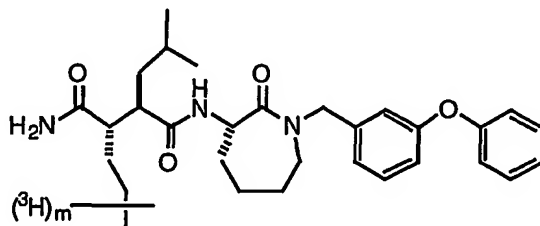
(II)

wherein:

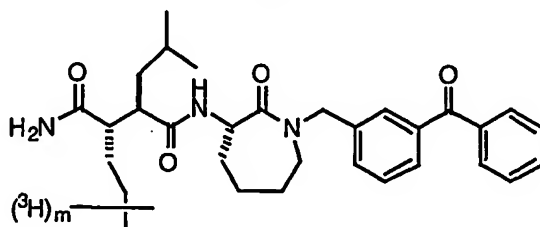
at least one atom of the compound of the Formula (II) is radiolabeled.

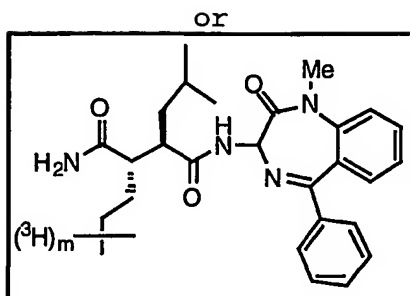
- 15 [10] In an even further more preferred embodiment the present invention, provides a method wherein R^3 , in a compound of Formula (II), is $\text{C}_3\text{-C}_6$ alkyl substituted with about 1 to about 4 ^3H .

- 20 [11] In a most preferred embodiment the present invention, provides a method wherein the tagged inhibitor of beta-amyloid production comprises a compound of Formula:



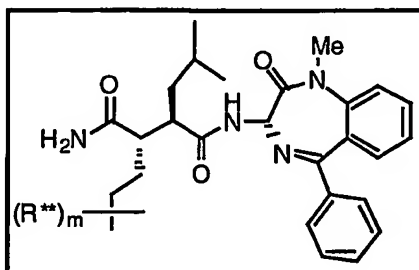
or





wherein m is about 2.

- 5 [12] In a further most preferred embodiment the present invention, provides a method wherein the tagged inhibitor of beta-amyloid production comprises a compound of Formula (I-43T)



(I-43T)

wherein m is about 2.

- [] In yet another preferred embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a compound selected from
 15 US 5,703,129; PCT application WO98/28268; PCT application WO98/22441; PCT application WO98/22433; PCT application WO98/22430; PCT application WO98/22493; PCT application WO98/22494; PCT application WO98/38177; or PCT application
 20 WO95/09838; wherein the compound has been tagged for purposes of the invention.

- [13] In another preferred embodiment the present invention provides a method wherein at least one macromolecule
 25 involved in the processing of APP and the production of beta-amyloid peptide comprises presenilin 1 or a fragment of presenilin 1.

[14] In another preferred embodiment the present invention provides a method wherein the macromolecule involved in the processing of APP and/or the production of beta-amyloid peptide comprises:

- 5 (1) presenilin-1;
- (2) presenilin-2;
- (3) β secretase;
- (4) α secretase;
- (5) γ secretase; or
- 10 (6) BACE/memapsin 2;

or any fragment or derivative thereof.

[] In another preferred embodiment the present invention
15 provides a method wherein at least one macromolecule involved in the processing of APP and the production of beta-amyloid peptide comprises either 1) presenilin 1 or a fragment of presenilin 1 or 2) presenilin 2 or a fragment of presenilin 2; but not both.

20

[15] In yet another preferred embodiment the present invention provides a method wherein the inhibitory concentration is half maximal inhibitory concentration.

25 [16] In a second embodiment, the present invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of an inhibitor of beta-amyloid production identified by the screening assay of Claim 1 or a pharmaceutically
30 acceptable salt or prodrug form thereof.

[17] In a third embodiment, the present invention provides a method for treating degenerative neurological disorders involving beta-amyloid production comprising administering
35 to a host in need of such treatment a therapeutically effective amount of an inhibitor of beta-amyloid production identified by the screening assay of Claim 1 or a pharmaceutically acceptable salt or prodrug form thereof.

[18] In a preferred third embodiment the degenerative neurological disorder is Alzheimer's Disease.

5 [19] In a fourth embodiment, the present invention provides a method of identifying a macromolecule involved in APP processing comprising

- 1) contacting a tagged inhibitor of beta-amyloid production with material suspected to contain a
10 macromolecule involved in APP processing;
- 2) separating a complex comprising a tagged inhibitor of beta-amyloid production and a macromolecule involved in APP processing; and
- 3) identifying the complex.

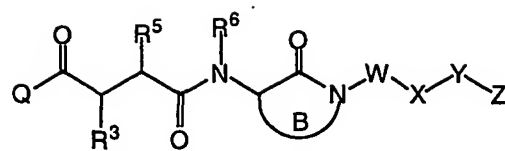
15

[20] In a preferred fourth embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a radiolabeled inhibitor of beta-amyloid production, a fluorescence labeled inhibitor
20 of beta-amyloid production, a biotin labeled inhibitor of beta-amyloid production, a photoaffinity labeled inhibitor of beta-amyloid production, or any combination of tags thereof in one inhibitor of beta-amyloid production.

25 [21] In a preferred fourth embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a radiolabeled inhibitor of beta-amyloid production.

30 [22] In a more preferred fourth embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a tritium labeled inhibitor of beta-amyloid production.

35 [23] In a more preferred fourth embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a compound of Formula (I):



(I)

wherein:

- 5 at least one atom of the compound of the Formula (I) is radiolabeled;

Q is -NR¹R²;

- 10 R¹, at each occurrence, is independently selected from:
 H;
 C₁-C₆ alkyl substituted with 0-3 R^{1a};
 C₃-C₁₀ carbocycle substituted with 0-3 R^{1b};
 C₆-C₁₀ aryl substituted with 0-3 R^{1b}; and
 15 5 to 10 membered heterocycle substituted with 0-3 R^{1b};

R^{1a}, at each occurrence, is independently selected from H,
 C₁-C₆ alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶,
 phenyl, CF₃;

- 20 C₃-C₁₀ carbocycle substituted with 0-3 R^{1b};
 C₆-C₁₀ aryl substituted with 0-3 R^{1b}; and
 5 to 10 membered heterocycle substituted with 0-3 R^{1b};

- R^{1b}, at each occurrence, is independently selected from H,
 25 OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂,
 NR¹⁵R¹⁶, or CF₃;

- R² is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₆
 alkoxy, C₃-C₁₀ carbocycle, C₆-C₁₀ aryl and 5 to 10
 30 membered heterocycle;

R³ is C₁-C₆ alkyl substituted with 0-1 R⁴;

- R⁴ is H, OH, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆
 35 alkynyl, C₃-C₁₀ carbocycle, C₆-C₁₀ aryl, or 5 to 10
 membered heterocycle;

R⁵ is H, OR¹⁴;

C₁-C₆ alkyl substituted with 0-3 R^{5b};

C₁-C₆ alkoxy substituted with 0-3 R^{5b};

5 C₂-C₆ alkenyl substituted with 0-3 R^{5b};

C₂-C₆ alkynyl substituted with 0-3 R^{5b};

C₃-C₁₀ carbocycle substituted with 0-3 R^{5c};

C₆-C₁₀ aryl substituted with 0-3 R^{5c}; or

5 to 10 membered heterocycle substituted with 0-3 R^{5c};

10

R^{5b}, at each occurrence, is independently selected from:

H, C₁-C₆ alkyl, CF₃, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂,

NR¹⁵R¹⁶;

C₃-C₁₀ carbocycle substituted with 0-3 R^{5c};

15 C₆-C₁₀ aryl substituted with 0-3 R^{5c}; or

5 to 10 membered heterocycle substituted with 0-3 R^{5c};

R^{5c}, at each occurrence, is independently selected from H,

OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂,

20 NR¹⁵R¹⁶, or CF₃;

R⁶ is H;

C₁-C₆ alkyl substituted with 0-3 R^{6a};

C₃-C₁₀ carbocycle substituted with 0-3 R^{6b}; or

25 C₆-C₁₀ aryl substituted with 0-3 R^{6b};

R^{6a}, at each occurrence, is independently selected from H,

C₁-C₆ alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶,

phenyl or CF₃;

30

R^{6b}, at each occurrence, is independently selected from H,

OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂,

NR¹⁵R¹⁶, or CF₃;

35 W is -(CR⁸R^{8a})_p-;

p is 0 to 4;

R⁸ and R^{8a}, at each occurrence, are independently selected from H, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₂-C₄ alkynyl and C₃-C₈ cycloalkyl;

5 X is a bond;
C₆-C₁₀ aryl substituted with 0-3 R^{Xb};
C₃-C₁₀ carbocycle substituted with 0-3 R^{Xb}; or
5 to 10 membered heterocycle substituted with 0-3 R^{Xb};

10 R^{Xb}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;

Y is a bond or -(CR⁹R^{9a})_t-V-(CR⁹R^{9a})_u-;

15

t is 0 to 3;

u is 0 to 3;

20 R⁹ and R^{9a}, at each occurrence, are independently selected from H, C₁-C₆ alkyl or C₃-C₈ cycloalkyl;

V is a bond, -C(=O)-, -O-, -S-, -S(=O)-, -S(=O)₂-, -N(R¹⁹)-, -C(=O)NR^{19b}-, -NR^{19b}C(=O)-, -NR^{19b}S(=O)₂-, -S(=O)₂NR^{19b}-,
25 , -NR^{19b}S(=O)-, -S(=O)NR^{19b}-, -C(=O)O-, or -OC(=O)-;

Z is H;

C₁-C₈ alkyl substituted with 0-2 R¹²;
C₂-C₄ alkenyl substituted with 0-2 R¹²;
30 C₂-C₄ alkynyl substituted with 0-2 R¹²;
C₆-C₁₀ aryl substituted with 0-4 R^{12b};
C₃-C₁₀ carbocycle substituted with 0-4 R^{12b}; or
5 to 10 membered heterocycle substituted with 0-3 R^{12b};
35 R¹² is C₆-C₁₀ aryl substituted with 0-4 R^{12b};
C₃-C₁₀ carbocycle substituted with 0-4 R^{12b}; or
5 to 10 membered heterocycle substituted with 0-3 R^{12b};

R^{12b}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;

5 B is a 5 to 10 membered lactam, wherein the lactam is saturated, partially saturated or unsaturated; wherein each additional lactam carbon is substituted with 0-2 R¹¹; and, optionally, the lactam contains a heteroatom selected from -O-, -S-, -S(=O)-, -S(=O)₂-, -N=, and -
10 N(R¹⁰)-;

R¹⁰ is H, C(=O)R¹⁷, C(=O)OR¹⁷, C(=O)NR¹⁸R¹⁹, S(=O)₂NR¹⁸R¹⁹, S(=O)₂R¹⁷;

C₁-C₆ alkyl optionally substituted with R^{10a};
15 C₆-C₁₀ aryl substituted with 0-4 R^{10b};
C₃-C₁₀ carbocycle substituted with 0-3 R^{10b}; or
5 to 10 membered heterocycle optionally substituted with 0-3 R^{10b};

20 R^{10a}, at each occurrence, is independently selected from H, C₁-C₆ alkyl, C₃-C₆ cycloalkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶, phenyl or CF₃;

R^{10b}, at each occurrence, is independently selected from H,
25 OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;

R¹¹ is C₁-C₄ alkoxy, Cl, F, Br, I, =O, CN, NO₂, NR¹⁸R¹⁹, C(=O)R¹⁷, C(=O)OR¹⁷, C(=O)NR¹⁸R¹⁹, S(=O)₂NR¹⁸R¹⁹, CF₃;

30 C₁-C₆ alkyl optionally substituted with R^{11a};
C₆-C₁₀ aryl substituted with 0-3 R^{11b};
C₃-C₁₀ carbocycle substituted with 0-3 R^{11b}; or
5 to 10 membered heterocycle substituted with 0-3 R^{11b};

35 alternatively, two R¹¹ substituents on the same carbon atoms may be combined to form a C₃-C₆ carbocycle;

alternatively, two R¹¹ substituents on adjacent carbon atoms may be combined to form a C₃-C₆ carbocycle or a benzo fused radical, wherein said benzo fused radical is substituted with 0-3 R¹³;

5

R^{11a}, at each occurrence, is independently selected from H, C₁-C₆ alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶, phenyl or CF₃;

10 R^{11b}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;

15 R¹³, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;

R¹⁴ is H, phenyl, benzyl, C₁-C₆ alkyl, or C₂-C₆ alkoxyalkyl;

20 R¹⁵, at each occurrence, is independently selected from H, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);

25 R¹⁶, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);

R¹⁷ is H, phenyl, benzyl, C₁-C₆ alkyl, or C₂-C₆ alkoxyalkyl;

30 R¹⁸, at each occurrence, is independently selected from H, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl); and

35 R¹⁹, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, phenyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);

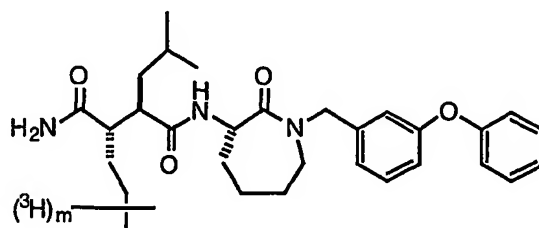
R^{19b} is H, C₁-C₆ alkyl, C₃-C₈ cycloalkyl, phenyl, benzyl or phenethyl; and

R²⁰ is H or C₁-C₆ alkyl.

5

[24] In an even more preferred fourth embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a compound of the Formula (I-7T):

10

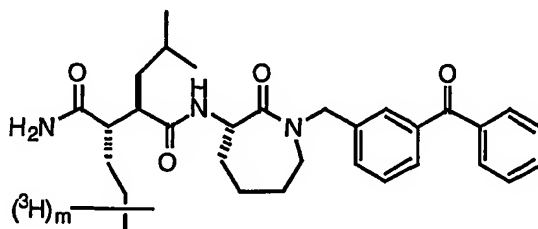


(I-7T)

wherein m is about 2.

15 [26] In another preferred fourth embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production is radiolabeled and photoaffinity labeled.

20 [27] In a more preferred fourth embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a compound of the Formula (I-11T):



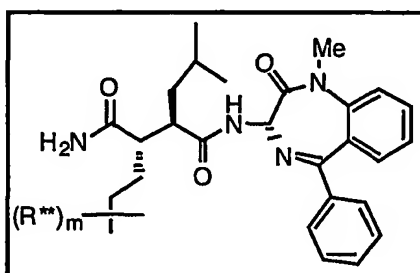
(I-11T)

wherein m is about 2.

25

[] In an even further more preferred fourth embodiment the present invention provides a method wherein the tagged inhibitor of beta-amyloid production comprises a compound of the Formula (I-43T):

5



(I-43T)

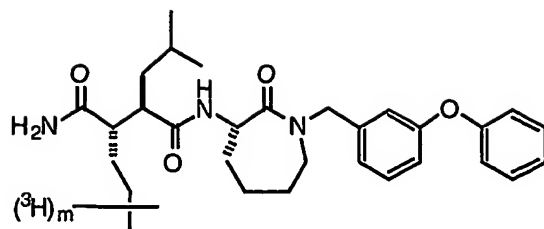
wherein m is about 2.

10 [28] In fifth embodiment the present invention provides a macromolecule involved in APP processing which a tagged inhibitor of beta-amyloid production binds to specifically.

15 [29] In a preferred fifth embodiment the present invention provides a macromolecule wherein the the tagged inhibitor of beta-amyloid production comprises a radiolabeled inhibitor of beta-amyloid production, a fluorescence labeled inhibitor of beta-amyloid production, a biotin labeled inhibitor of beta-amyloid production, a
20 photoaffinity labeled inhibitor of beta-amyloid production, or any combination of tags thereof in one inhibitor of beta-amyloid production.

25 [30] In a preferred fifth embodiment the present invention provides a macromolecule wherein the tagged inhibitor of beta-amyloid production comprises a radiolabeled inhibitor of beta-amyloid production.

30 [31] In a more preferred fifth embodiment the present invention provides a macromolecule wherein the tagged inhibitor of beta-amyloid production comprises a compound of the Formula (I-7T):



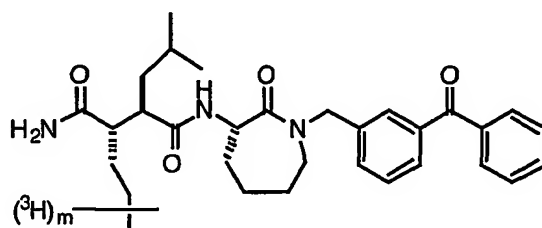
(I-7T)

wherein m is about 2.

5

[32] In another preferred fifth embodiment the present invention provides a macromolecule wherein the tagged inhibitor of beta-amyloid production comprises a compound of the Formula (I-11T):

10



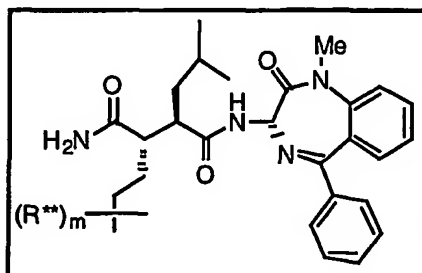
(I-11T)

wherein m is about 2.

15

[33] In another preferred fifth embodiment the present invention provides a macromolecule wherein the tagged inhibitor of beta-amyloid production comprises a compound of the Formula (I-43T):

20



(I-43T)

wherein m is about 2.

[34] In another preferred fifth embodiment the present invention provides a macromolecule involved in APP processing which macromolecule is presenilin 1 or a fragment of presenilin 1.

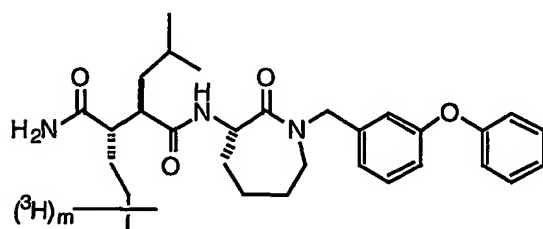
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[35] In another preferred fifth embodiment the present invention provides a macromolecule involved in APP processing which macromolecule is presenilin 2 or a fragment of presenilin 2.

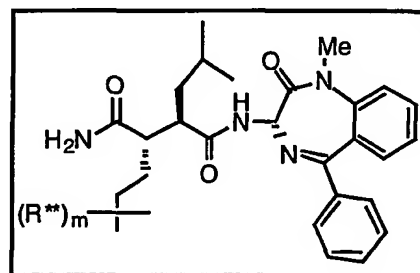
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[36] In a sixth embodiment the present invention provides an inhibitor of beta-amyloid production comprising a compound which interacts with a binding site on a macromolecule involved in the production of beta-amyloid peptide; wherein said binding site is identified by a compound of Formula (I-7T) or (I-43T):

15



(I-7T)



(I-43T)

20

wherein m is about 2.

[36] In the sixth embodiment the binding site is identified as a specific binding site for a compound of Formula (I-7T) or (I-43T), wherein m is about 2.

25

[37] In a preferred sixth embodiment the macromolecule involved in the production of beta-amyloid peptide is presenilin 1 or a fragment of presenilin 1.

30

[38] In a preferred sixth embodiment the macromolecule involved in the production of beta-amyloid peptide is presenilin 2 or a fragment of presenilin 2.

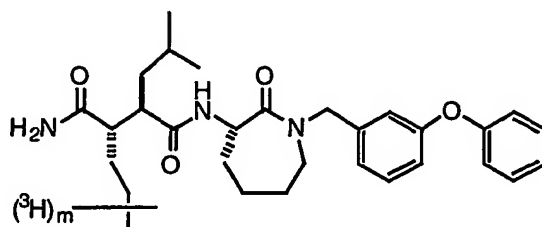
[39] In another preferred sixth embodiment the invention provides an inhibitor of beta-amyloid production comprising a compound which interacts with a binding site on a
5 macromolecule involved in the production of beta-amyloid peptide; wherein said binding site is a specific binding site for a compound of Formula (I-7T), wherein m is about 2; and the compound demonstrates a half maximal inhibitory concentration less than 10 micromolar for beta-amyloid
10 production.

[40] In a more preferred sixth embodiment the invention provides an inhibitor of beta-amyloid production comprising a compound which interacts with a binding site on
15 presenilin 1 or a fragment of presenilin 1; wherein said binding site is a specific binding site for a compound of Formula (I-7T), wherein m is about 2; and the compound demonstrates a half maximal inhibitory concentration less than 10 micromolar for beta-amyloid production.

20 [41] In another preferred sixth embodiment the invention provides an inhibitor of beta-amyloid production comprising a compound which interacts with a binding site on a macromolecule involved in the production of beta-amyloid
25 peptide; wherein said binding site is a specific binding site for a compound of Formula (I-43T), wherein m is about 2; and the compound demonstrates a half maximal inhibitory concentration less than 10 micromolar for beta-amyloid production.

30 [42] In another more preferred sixth embodiment the invention provides an inhibitor of beta-amyloid production comprising a compound which interacts with a binding site on presenilin 1 or a fragment of presenilin 1; wherein said
35 binding site is a specific binding site for a compound of Formula (I-43T), wherein m is about 2; and the compound demonstrates a half maximal inhibitory concentration less than 10 micromolar for beta-amyloid production.

[43] In a seventh embodiment the present invention provides a tagged inhibitor of beta-amyloid production comprising a tagged compound which interacts with a binding site on a macromolecule involved in the production of beta-amyloid peptide; wherein said binding site is identified by a compound of Formula (I-7T):



(I-7T)

wherein m is about 2;

[43] In the seventh embodiment the binding site is identified as a specific binding site for a compound of Formula (I-7T) or (I-43T), wherein m is about 2.

[44] In a preferred seventh embodiment the macromolecule involved in the production of beta-amyloid peptide is presenilin 1 or a fragment of presenilin 1.

[45] In a preferred seventh embodiment the macromolecule involved in the production of beta-amyloid peptide is presenilin 2 or a fragment of presenilin 2.

[46] In another preferred seventh embodiment the invention provides a tagged inhibitor of beta-amyloid production comprising a tagged compound which interacts with a binding site on a macromolecule involved in the production of beta-amyloid peptide; wherein said binding site is a specific binding site for a compound of Formula (I-7T), wherein m is about 2; and the tagged compound demonstrates a half

maximal inhibitory concentration less than 10 micromolar for beta-amyloid production.

[47] In a more preferred seventh embodiment the invention
5 provides a tagged inhibitor of beta-amyloid production
comprising a tagged compound which interacts with a binding
site on presenilin 1 or a fragment of presenilin 1; wherein
said binding site is a specific binding site for a compound
of Formula (I-7T), wherein m is about 2; and the tagged
10 compound demonstrates a half maximal inhibitory
concentration less than 10 micromolar for beta-amyloid
production.

[48] In another preferred seventh embodiment the invention
15 provides a tagged inhibitor of beta-amyloid production
comprising a tagged compound which interacts with a binding
site on a macromolecule involved in the production of beta-
amyloid peptide; wherein said binding site is a specific
binding site for a compound of Formula (I-43T), wherein m
20 is about 2; and the tagged compound demonstrates a half
maximal inhibitory concentration less than 10 micromolar
for beta-amyloid production.

[49] In another more preferred seventh embodiment the
25 invention provides a tagged inhibitor of beta-amyloid
production comprising a tagged compound which interacts
with a binding site on presenilin 1 or a fragment of
presenilin 1; wherein said binding site is a specific
binding site for a compound of Formula (I-43T), wherein m
30 is about 2; and the tagged compound demonstrates a half
maximal inhibitory concentration less than 10 micromolar
for beta-amyloid production.

[] In yet another preferred embodiment the present
35 invention provides a tagged inhibitor of beta-amyloid
production comprising a compound claimed in or within the
scope of compounds claimed in a reference selected from
Table 1:

Table 1

US 5,703,129;

PCT application WO98/28268;

- 5 PCT application WO98/22441;
PCT application WO98/22433;
PCT application WO98/22430;
PCT application WO98/22493;
PCT application WO98/22494;
- 10 PCT application WO98/38177; and
PCT application WO95/09838;
wherein the compound has been tagged for purposes of the
invention.
- 15 [] In an eighth embodiment the present invention provides
a use of a macromolecule or complex of macromolecules
involved in APP processing, which a tagged inhibitor of
beta-amyloid production binds to specifically, for the
identification or assaying of inhibitors as therapeutics
- 20 for neurological and other disorders involved in APP
processing and beta-amyloid production.
- [] In a preferred eighth embodiment the present invention
provides a use of a macromolecule or complex of
- 25 macromolecules involved in APP processing, which
macromolecule or complex of macromolecules is presenilin 1
or a fragment of presenilin 1.
- [50] In a more preferred eighth embodiment the present
- 30 invention provides a method of identifying inhibitors as
therapeutics for neurological and other disorders involved
in APP processing and beta-amyloid production comprising
- (1) contacting at least one macromolecule involved in
APP processing and beta-amyloid production, which
- 35 macromolecule a tagged inhibitor of beta-amyloid production
binds to specifically, with a potential inhibitor of beta-
amyloid production; and

(2) determining the level of inhibition of APP processing and beta-amyloid production.

5 [51] In an even more preferred eighth embodiment the present invention provides a method wherein the macromolecule is a complex of macromolecules.

10 [52] In an even more preferred eighth embodiment the present invention provides a method of wherein the macromolecule is presenilin 1 or a fragment of presenilin 1.

15 [53] In an even more preferred eighth embodiment the present invention provides a method of wherein the macromolecule is presenilin 2 or a fragment of presenilin 2.

20 [54] In a ninth embodiment the present invention provides a method of treating Alzheimer's disease comprising administering to a host in need of such treatment a therapeutically effective amount of an inhibitor of beta-amyloid production, or a pharmaceutically acceptable salt or prodrug form thereof, wherein said inhibitor of beta-amyloid production binds to a binding site on a
25 macromolecule involved in the production of beta-amyloid peptide and effects a decrease in production of beta-amyloid peptide;

wherein said binding site is a specific binding site for a compound of Formula (I-7T) or (I-43T) wherein m is about 2.
30

[55] In a preferred ninth embodiment the macromolecule comprises presenilin-1, a fragment of presenilin-1, presenilin-2, or a fragment of presenilin-2.

35 [56] In another preferred ninth embodiment the binding site is a specific binding site for a compound of Formula (I-43T) wherein m is about 2.

[57] In a more preferred ninth embodiment the macromolecule comprises presenilin-1 or a fragment of presenilin-1.

[58] In another more preferred ninth embodiment the
5 macromolecule comprises presenilin-2 or a fragment of presenilin-2.

[58a] The present invention also provides radiolabeled inhibitors of APP processing and/or the production of
10 beta-amyloid production for use in methods of in vivo diagnostic imaging in the diagnosis of diseases involving APP processing and/or the production of beta-amyloid production. Also provided in the present invention are methods of in vivo diagnostic imaging comprising
15 administering to a subject a diagnostically effective amount of a radiolabeled inhibitor of APP processing and/or the production of beta-amyloid production. As used herein, the term "radiolabeled inhibitors of APP processing" or "radiolabeled inhibitor of beta-amyloid production", when
20 applied to uses for in vivo diagnostic imaging, also includes any ligand that binds to a macromolecule involved in APP processing or beta-amyloid production with an affinity and selectivity suitable for in vivo diagnostic imaging. Thus, such suitable ligand does not need to be an
25 inhibitor of APP processing or beta-amyloid production. Although the radiolabeled ligand may be an inhibitor of beta-amyloid production when administered at therapeutic doses, the relatively low levels of radiolabeled ligand used in an in vivo diagnostic imaging procedure will
30 generally be well below such therapeutic levels. Thus, for purposes of in vivo diagnostic imaging, the term "radiolabeled inhibitor of APP processing" or "radiolabeled inhibitor of beta-amyloid production" refers to any suitable radiolabeled compound which binds in vivo to the
35 same macromolecule target as a compound that is an inhibitor of APP processing and/or beta-amyloid production as described herein.

[59] The present invention includes the above-described method of in vivo diagnostic imaging comprising administering to a subject a diagnostically effective amount of a radiolabeled inhibitor of beta-amyloid
5 production.

[60] The present invention includes the above-described method of in vivo diagnostic imaging wherein said method is used in the diagnosis of a neurological disease which
10 involves APP processing or elevated levels of beta-amyloid, or both.

[61] The present invention includes the above-described method of in vivo diagnostic imaging wherein said method is
15 used in the diagnosis of Alzheimer's disease.

[62] The present invention includes the above-described
20 method of in vivo diagnostic imaging wherein the radiolabeled inhibitor is suitable for imaging of the brain of the subject.

[63] The present invention includes the above-described
25 method of in vivo diagnostic imaging wherein the radiolabeled inhibitor is radiolabeled with one or more radioisotope selected from ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{123}I , ^{125}I , ^{131}I .

[64] The present invention includes the above-described
30 method of in vivo diagnostic imaging wherein wherein the inhibitor of beta-amyloid production is a compound selected from any compound claimed in or within the scope of compounds claimed in a reference selected from:

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- (1) United States patent US 5,703,129;
- (2) PCT application WO98/22441 (or its priority USSN 08/755,444);

- (3) PCT application WO98/22433 (or its priority USSN 08/807,538);
- (4) PCT application WO98/22430 (or its priority USSN 08/754,895);
- 5 (5) PCT application WO98/22493 (or its priority USSN 08/755,334);
- (6) PCT application WO98/22494 (or its priorities USSN 08/808,528, 08/807,528 or 08/807,427);
- (7) PCT application WO98/28268 (or its priority USSN 10 08/780,025);
- (8) PCT application WO98/38177;
- (9) PCT application WO95/09838;
- (10) PCT application WO99/67221;
- (11) PCT application WO99/67220;
- 15 (12) PCT application WO99/67219;
- (13) PCT application WO95/66934;
- (14) PCT application WO00/24392;
- (15) Ghosh et al., JACS (2000) 122:3522-2523;
- (16) PCT application No. US99/17717, filed Aug. 7, 1999
- 20 and US patent application No. USSN 09/370,089, filed Aug, 6, 1999 (now abandoned);
- (17) US patent application No. 09/506,360, filed Feb 17, 2000;
- (18) PCT application No. US99/30815, filed Dec 23, 1999
- 25 and the US patent application No. 09/469,939, filed Dec 24, 1999;
- (19) PCT application No. US00/24967 filed Sept 13, 2000 and the US patent application No. 09/661,008, filed Sept 13, 2000;
- 30 (20) PCT application No. US00/27666 filed Oct 7, 2000 and the US patent application No. 09/684,718, filed Oct. 7, 2000;
- (21) PCT application No. US01/05236 filed Feb 16, 2001 and the US patent application No. 09/788,227, filed Feb
- 35 16, 2001;
- (22) PCT application No. US01/09703 filed March 27, 2001 and the US patent application No. 09/817,957, filed March 27, 2001;

(23) PCT application No. US01/10297 filed March 30, 2001;
(24) PCT application No. US01/11714 filed Apr 11, 2001
and the US patent application No. 09/832,455, filed Apr
11, 2001;

5 (25) PCT application No. US01/10667 filed Apr 3, 2001 and
the US patent application No. 09/825,211, filed Apr 3,
2001; and

(26) PCT application No. US01/10773 filed Apr 3, 2001 and
the US patent application No. 09/824,945, filed Apr 3,
10 2001.

or any compound which inhibits beta-amyloid production and
binds competitively with any of the foregoing compounds in
any of the assays described in the Utility section hereof;
all of which foregoing references are hereby incorporated
15 by reference in their entirety.

[65] The present invention includes the above-described
method of in vivo diagnostic imaging wherein the inhibitor
of beta-amyloid production exhibits activity as an
20 inhibitor in any of the above-described methods [1]-[15]
above.

[66] The present invention includes the above-described
method of in vivo diagnostic imaging wherein the inhibitor
25 of beta-amyloid production binds to a macromolecule which
is capable of being identified by any of the above-
described methods [19]-[27] above.

[67] The present invention includes the above-described
30 method of in vivo diagnostic imaging wherein the inhibitor
of beta-amyloid production binds to a macromolecule as
described above in [28]-[35] above.

[68] The present invention includes the above-described
35 method of in vivo diagnostic imaging wherein the inhibitor
of beta-amyloid production is selected from any of the
above-described inhibitors in [36]-[42] above.

[69] The present invention includes the above-described method of in vivo diagnostic imaging wherein the radiolabeled inhibitor of beta-amyloid production is a radiolabeled tagged inhibitor as described above [43]-[50]
5 above.

[70] The present includes the above-described method of in vivo diagnostic imaging wherein the inhibitor of beta-amyloid production is selected from:

- 10 (1) an inhibitor of presenilin-1;
- (2) an inhibitor of presenilin-2;
- (3) an inhibitor of β secretase;
- (4) an inhibitor of α secretase;
- (5) an inhibitor of γ secretase; or
- 15 (6) an inhibitor of BACE/memapsin 2.

[71] The present invention also provides pharmaceutical compositions suitable for in vivo diagnostic imaging comprising a radiolabeled inhibitor of beta-amyloid
20 production. Included in the present invention are the above-described radiolabeled inhibitors of beta-amyloid production, wherein the radiolabel is selected from the group ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{123}I , ^{125}I , and ^{131}I .
Included in the present invention is a radiopharmaceutical
25 composition comprising a radiopharmaceutically acceptable carrier and a radiolabeled inhibitor of beta-amyloid production. Included in the present invention is a method of determining levels of proteins involved in beta-amyloid production in a mammal comprising administering to the
30 mammal a radiopharmaceutical composition comprising a radiolabeled inhibitor of beta-amyloid production, and imaging said mammal. Included in the present invention is a method of diagnosing a disorder associated with beta-amyloid production in a mammal comprising
35 administering to the mammal a radiopharmaceutical composition comprising a radiolabeled inhibitor of beta-amyloid production, and imaging said mammal.

[72] The present invention includes the above-described pharmaceutical compositions wherein the composition is used in the diagnosis of a neurological disease which involves APP processing or elevated levels of beta-amyloid, or both.

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[73] The present invention includes the above-described pharmaceutical compositions wherein the composition is used in the diagnosis of Alzheimer's disease.

10 [74] The present invention includes the above-described pharmaceutical compositions wherein the radiolabeled inhibitor is suitable for imaging of the brain of the subject.

15 [75] The present invention includes the above-described pharmaceutical compositions wherein the radiolabeled inhibitor is radiolabeled with one or more radioisotope selected from ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{123}I , ^{125}I , ^{131}I .

20 [76] The present invention includes the above-described pharmaceutical compositions wherein the inhibitor of beta-amyloid production is a compound selected from any compound claimed in or within the scope of compounds claimed in a reference selected from Table 2:

25

Table 2

- (1) United States patent US 5,703,129;
- (2) PCT application W098/22441 (or its priority USSN 08/755,444);
- (3) PCT application W098/22433 (or its priority USSN 30 08/807,538);
- (4) PCT application W098/22430 (or its priority USSN 08/754,895);
- (5) PCT application W098/22493 (or its priority USSN 08/755,334);
- 35 (6) PCT application W098/22494 (or its priorities USSN 08/808,528, 08/807,528 or 08/807,427);
- (7) PCT application W098/28268 (or its priority USSN 08/780,025);

- (8) PCT application WO98/38177;
(9) PCT application WO95/09838;
(10) PCT application WO99/67221;
(11) PCT application WO99/67220;
5 (12) PCT application WO99/67219;
(13) PCT application WO95/66934;
(14) PCT application WO00/24392;
(15) Ghosh et al., JACS (2000) 122:3522-2523;
(16) PCT application No. US99/17717, filed Aug. 7, 1999
10 and US patent application No. USSN 09/370,089, filed Aug,
6, 1999 (now abandoned);
(17) US patent application No. 09/506,360, filed Feb 17,
2000;
(18) PCT application No. US99/30815, filed Dec 23, 1999
15 and the US patent application No. 09/469,939, filed Dec
24, 1999;
(19) PCT application No. US00/24967 filed Sept 13, 2000
and the US patent application No. 09/661,008, filed Sept
13, 2000;
20 (20) PCT application No. US00/27666 filed Oct 7, 2000 and
the US patent application No. 09/684,718, filed Oct. 7,
2000;
(21) PCT application No. US01/05236 filed Feb 16, 2001
and the US patent application No. 09/788,227, filed Feb
25 16, 2001;
(22) PCT application No. US01/09703 filed March 27, 2001
and the US patent application No. 09/817,957, filed March
27, 2001;
(23) PCT application No. US01/10297 filed March 30, 2001;
30 (24) PCT application No. US01/11714 filed Apr 11, 2001
and the US patent application No. 09/832,455, filed Apr
11, 2001;
(25) PCT application No. US01/10667 filed Apr 3, 2001 and
the US patent application No. 09/825,211, filed Apr 3,
35 2001; and
(26) PCT application No. US01/10773 filed Apr 3, 2001 and
the US patent application No. 09/824,945, filed Apr 3,
2001.

or any compound which inhibits beta-amyloid production and binds competitively with any of the foregoing compounds in any of the assays described in the Utility section hereof;

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all of which foregoing references are hereby incorporated by reference in their entirety.

[77] The present includes the above-described
10 pharmaceutical composition wherein the inhibitor of beta-amyloid production is selected from:

- (1) an inhibitor of presenilin-1;
- (2) an inhibitor of presenilin-2;
- (3) an inhibitor of β secretase;
- 15 (4) an inhibitor of α secretase;
- (5) an inhibitor of γ secretase; or
- (6) an inhibitor of BACE/memapsin 2.

20 In a tenth embodiment the present invention provides a method for diagnosing a neurological disease involving APP processing and/or the production of beta-amyloid production within a mammalian body comprising:

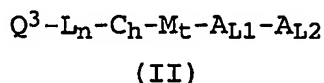
- 25 (a) administering a diagnostically effective amount of a radiopharmaceutical inhibitor of APP processing and/or the production of beta-amyloid production; and
- (b) imaging the area of the patient wherein the disease is located.

30 In a preferred aspect of the tenth embodiment the present invention provides a method wherein the radiopharmaceutical comprises technetium-99m, indium-111, or gallium-68.

35 In a preferred aspect of the tenth embodiment the present invention provides a method wherein the radiopharmaceutical comprises technetium-99m.

In a preferred aspect of the tenth embodiment the present invention provides a method wherein the radiopharmaceutical is a compound of Formula II:

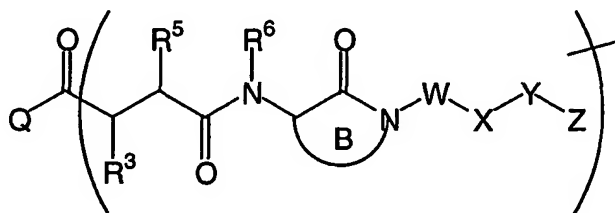
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wherein

- 10 Q^3 is an inhibitor of APP processing and/or an inhibitor of beta-amyloid production;
- L_n is a linking group;
- C_h is a radionuclide metal chelator coordinated to a transition metal radionuclide M_t ;
- M_t is a transition metal radionuclide;
- 15 A_{L1} is a first ancillary ligand; and
- A_{L2} is a second ancillary ligand capable of stabilizing the radiopharmaceutical;
- and pharmaceutically acceptable salts thereof.

- 20 In a preferred aspect of the tenth embodiment the present invention provides a method wherein Q^3 is a radical of a compound of formula (I):



25

(I)

wherein:

- Q is $-NH_2$;
- R^3 is C_1 - C_6 alkyl substituted with 0-1 R^4 ;
- R^4 is H, OH, C_1 - C_6 alkoxy, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_{10} carbocycle, C_6 - C_{10} aryl, or 5 to 10 membered heterocycle;
- 30 R^5 is H, OR¹⁴;
- C_1 - C_6 alkyl substituted with 0-3 R^{5b} ;
- C_1 - C_6 alkoxy substituted with 0-3 R^{5b} ;

- C2-C6 alkenyl substituted with 0-3 R^{5b};
 C2-C6 alkynyl substituted with 0-3 R^{5b};
 C3-C10 carbocycle substituted with 0-3 R^{5c};
 C6-C10 aryl substituted with 0-3 R^{5c}; or
 5 5 to 10 membered heterocycle substituted with 0-3 R^{5c};
 R^{5b}, at each occurrence, is independently selected from:
 H, C1-C6 alkyl, CF₃, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂,
 NR¹⁵R¹⁶;
 C3-C10 carbocycle substituted with 0-3 R^{5c};
 10 C6-C10 aryl substituted with 0-3 R^{5c}; or
 5 to 10 membered heterocycle substituted with 0-3 R^{5c};
 R^{5c}, at each occurrence, is independently selected from H,
 OH, C1-C6 alkyl, C1-C4 alkoxy, Cl, F, Br, I, CN, NO₂,
 NR¹⁵R¹⁶, or CF₃;
 15 R⁶ is H;
 C1-C6 alkyl substituted with 0-3 R^{6a};
 C3-C10 carbocycle substituted with 0-3 R^{6b}; or
 C6-C10 aryl substituted with 0-3 R^{6b};
 R^{6a}, at each occurrence, is independently selected from H,
 20 C1-C6 alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶,
 phenyl or CF₃;
 R^{6b}, at each occurrence, is independently selected from H,
 OH, C1-C6 alkyl, C1-C4 alkoxy, Cl, F, Br, I, CN, NO₂,
 NR¹⁵R¹⁶, or CF₃;
 25 W is -(CR⁸R^{8a})_p-;
 p is 0 to 4;
 R⁸ and R^{8a}, at each occurrence, are independently selected
 from H, C1-C4 alkyl, C2-C4 alkenyl, C2-C4 alkynyl and
 C3-C8 cycloalkyl;
 30 X is a bond;
 C6-C10 aryl substituted with 0-3 R^{Xb};
 C3-C10 carbocycle substituted with 0-3 R^{Xb}; or
 5 to 10 membered heterocycle substituted with 0-3 R^{Xb};
 R^{Xb}, at each occurrence, is independently selected from H,
 35 OH, C1-C6 alkyl, C1-C4 alkoxy, Cl, F, Br, I, CN, NO₂,
 NR¹⁵R¹⁶, or CF₃;
 Y is a bond or -(CR⁹R^{9a})_t-V-(CR⁹R^{9a})_u-;

- t is 0 to 3;
u is 0 to 3;
R⁹ and R^{9a}, at each occurrence, are independently selected from H, C₁-C₆ alkyl or C₃-C₈ cycloalkyl;
- 5 V is a bond, -C(=O)-, -O-, -S-, -S(=O)-, -S(=O)₂-, -N(R¹⁹)-, -C(=O)NR^{19b}-, -NR^{19b}C(=O)-, -NR^{19b}S(=O)₂-, -S(=O)₂NR^{19b}-, -NR^{19b}S(=O)-, -S(=O)NR^{19b}-, -C(=O)O-, or -OC(=O)-;
- Z is H;
- 10 C₁-C₈ alkyl substituted with 0-2 R¹²;
C₂-C₄ alkenyl substituted with 0-2 R¹²;
C₂-C₄ alkynyl substituted with 0-2 R¹²;
C₆-C₁₀ aryl substituted with 0-4 R^{12b};
C₃-C₁₀ carbocycle substituted with 0-4 R^{12b}; or
- 15 5 to 10 membered heterocycle substituted with 0-3 R^{12b};
- R¹² is C₆-C₁₀ aryl substituted with 0-4 R^{12b};
C₃-C₁₀ carbocycle substituted with 0-4 R^{12b}; or
5 to 10 membered heterocycle substituted with 0-3 R^{12b};
- 20 R^{12b}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;
- B is a 5 to 10 membered lactam, wherein the lactam is
- 25 saturated, partially saturated or unsaturated; wherein each additional lactam carbon is substituted with 0-2 R¹¹; and, optionally, the lactam contains a heteroatom selected from -O-, -S-, -S(=O)-, -S(=O)₂-, -N= and -N(R¹⁰)-;
- 30 R¹⁰ is H, C(=O)R¹⁷, C(=O)OR¹⁷, C(=O)NR¹⁸R¹⁹, S(=O)₂NR¹⁸R¹⁹, S(=O)₂R¹⁷;
C₁-C₆ alkyl optionally substituted with R^{10a};
C₆-C₁₀ aryl substituted with 0-4 R^{10b};
C₃-C₁₀ carbocycle substituted with 0-3 R^{10b}; or
- 35 5 to 10 membered heterocycle optionally substituted with 0-3 R^{10b};

- R^{10a}, at each occurrence, is independently selected from H, C₁-C₆ alkyl, C₃-C₆ cycloalkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶, phenyl or CF₃;
- R^{10b}, at each occurrence, is independently selected from H,
5 OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;
- R¹¹ is C₁-C₄ alkoxy, Cl, F, Br, I, =O, CN, NO₂, NR¹⁸R¹⁹, C(=O)R¹⁷, C(=O)OR¹⁷, C(=O)NR¹⁸R¹⁹, S(=O)₂NR¹⁸R¹⁹, CF₃; C₁-C₆ alkyl optionally substituted with R^{11a};
10 C₆-C₁₀ aryl substituted with 0-3 R^{11b};
C₃-C₁₀ carbocycle substituted with 0-3 R^{11b}; or
5 to 10 membered heterocycle substituted with 0-3 R^{11b};
alternatively, two R¹¹ substituents on the same carbon atoms may be combined to form a C₃-C₆ carbocycle;
- 15 alternatively, two R¹¹ substituents on adjacent carbon atoms may be combined to form a C₃-C₆ carbocycle or a benzo fused radical, wherein said benzo fused radical is substituted with 0-3 R¹³;
- R^{11a}, at each occurrence, is independently selected from H,
20 C₁-C₆ alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶, phenyl or CF₃;
- R^{11b}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;
- 25 R¹³, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;
- R¹⁴ is H, phenyl, benzyl, C₁-C₆ alkyl, or C₂-C₆ alkoxyalkyl;
- 30 R¹⁵, at each occurrence, is independently selected from H, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);
- R¹⁶, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);
35
- R¹⁷ is H, phenyl, benzyl, C₁-C₆ alkyl, or C₂-C₆ alkoxyalkyl;

R¹⁸, at each occurrence, is independently selected from H, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl); and

5 R¹⁹, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, phenyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);

R^{19b} is H, C₁-C₆ alkyl, C₃-C₈ cycloalkyl, phenyl, benzyl or phenethyl; and

R²⁰ is H or C₁-C₆ alkyl.

10

In a preferred aspect of the tenth embodiment the present invention provides a method wherein Q³ is an inhibitor of beta-amyloid production selected from the group consisting of:

- 15 (1) an inhibitor of presenilin-1;
(2) an inhibitor of presenilin-2;
(3) an inhibitor of β secretase;
(4) an inhibitor of α secretase;
(5) an inhibitor of γ secretase; and
20 (6) an inhibitor of BACE/memapsin 2.

In a preferred aspect of the tenth embodiment the present invention provides a method wherein Q³ is an inhibitor of beta-amyloid production which is identified by
25 the method of screening for inhibitors of beta-amyloid production comprising,

- 30 1) contacting a potential inhibitor of beta-amyloid production and a tagged inhibitor of beta-amyloid production with at least one macromolecule involved in the processing of APP and the production of beta-amyloid peptide, said macromolecule containing a binding site specific for said tagged inhibitor of beta-amyloid production;
35 2) separating the tagged inhibitor of beta-amyloid production bound to said macromolecule from the tagged inhibitor of beta-amyloid production free from said macromolecule; and

- 3) determining an inhibitory concentration of the potential inhibitor of beta-amyloid production from the concentration of tagged inhibitor of beta-amyloid production bound to said macromolecule.

5

In a preferred aspect of the tenth embodiment the present invention provides a method wherein L_n is a linking group of about 5 Angstroms to about 10,000 Angstroms in length.

10

In a preferred aspect of the tenth embodiment the present invention provides a method wherein L_n is linking group of the formula $-M^{31}-Y^{31}(CR^{31}R^{32})_f(Z^1)_fY^{32}-M^{32}-$; wherein

15 M^{31} is $-(CH_2)_gZ^{31}]_{g'}-(CR^{31}R^{32})_{g''}-$;

M^{32} is $-(CR^{31}R^{32})_{g''}-[Z^{31}(CH_2)_g]_{g'}-$;

g is independently 0-10;

g' is independently 0-1;

g'' is independently 0-10;

20 f is independently 0-10;

f' is independently 0-10;

f'' is independently 0-1;

Y^{31} and Y^{32} , at each occurrence, are independently selected

from: a direct bond, $-O-$, $-NR^{32}-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)O-$, $-C(=O)NH-$, $-C(=NR^{32})-$, $-S-$, $-SO-$, $-SO_2-$, $-SO_3-$, $-NHC(=O)-$, $-(NH)_2C(=O)-$, $-(NH)_2C=S-$;

25 Z^{31} is independently selected at each occurrence from a (C_6-C_{14}) saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{33} ; and a heterocyclic ring system, optionally substituted with 0-4 R^{33} ;

30 R^{31} and R^{32} are independently selected at each occurrence from: hydrogen; (C_1-C_{10})alkyl substituted with 0-5 R^{33} ; alkaryl wherein the aryl is substituted with 0-5 R^{33} ;

35 R^{33} is independently selected at each occurrence from the group: hydrogen, $-OH$, $-NHR^{34}$, $-C(=O)R^{34}$, $-OC(=O)R^{34}$, $-OC(=O)OR^{34}$, $-C(=O)OR^{34}$, $-C(=O)NR^{34}$, $-CN$, $-SR^{34}$, $-SOR^{34}$,

$-\text{SO}_2\text{R}^{34}$, $-\text{NHC}(=\text{O})\text{R}^{34}$, $-\text{NHC}(=\text{O})\text{NHR}^{34}$, or $-\text{NHC}(=\text{S})\text{NHR}^{34}$;

and

R^{34} is independently selected at each occurrence from the group: hydrogen; $(\text{C}_1\text{-C}_6)\text{alkyl}$; benzyl, and phenyl.

5

In a preferred aspect of the tenth embodiment the present invention provides a method wherein:

L_n is a linking group of the formula $-\text{R}^{35}\text{-G-R}^{36}-$;

R^{35} and R^{36} are each independently $-\text{N}(\text{R}^{37})\text{C}(=\text{O})-$,

10 $-\text{C}(=\text{O})\text{N}(\text{R}^{37})-$, $-\text{OC}(=\text{O})-$, $-\text{C}(=\text{O})\text{O}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}(\text{O})-$, $-\text{SO}_2-$, $-\text{NR}^{37}-$, $-\text{C}(=\text{O})-$, or a direct bond;

each R^{37} is independently H or $(\text{C}_1\text{-C}_6)\text{alkyl}$;

G is $(\text{C}_1\text{-C}_{24})\text{alkyl}$ substituted with 0-3 R^{38} , cycloalkyl

substituted with 0-3 R^{38} , aryl substituted with 0-3

15 R^{38} , or heterocycle substituted with 0-3 R^{38} ;

R^{38} is $=\text{O}$, F, Cl, Br, I, $-\text{CF}_3$, $-\text{CN}$, $-\text{CO}_2\text{R}^{39}$, $-\text{C}(=\text{O})\text{R}^{39}$, $-\text{C}(=\text{O})\text{N}(\text{R}^{39})_2$, $-\text{CHO}$, $-\text{CH}_2\text{OR}^{39}$, $-\text{OC}(=\text{O})\text{R}^{39}$, $-\text{OC}(=\text{O})\text{OR}^{40}$, $-\text{OR}^{39}$, $-\text{OC}(=\text{O})\text{N}(\text{R}^{39})_2$, $-\text{NR}^{39}\text{C}(=\text{O})\text{R}^{39}$, $-\text{NR}^{41}\text{C}(=\text{O})\text{OR}^{40}$, $-\text{NR}^{39}\text{C}(=\text{O})\text{N}(\text{R}^{39})_2$, $-\text{NR}^{39}\text{SO}_2\text{N}(\text{R}^{39})_2$, $-\text{NR}^{41}\text{SO}_2\text{R}^{40}$, $-\text{SO}_3\text{H}$, $-\text{SO}_2\text{R}^{40}$, $-\text{SR}^{39}$, $-\text{S}(=\text{O})\text{R}^{40}$, $-\text{SO}_2\text{N}(\text{R}^{39})_2$, $-\text{N}(\text{R}^{39})_2$, $-\text{NHC}(=\text{NH})\text{NHR}^{39}$, $-\text{C}(=\text{NH})\text{NHR}^{39}$, $=\text{NOR}^{39}$, $-\text{NO}_2$, $-\text{C}(=\text{O})\text{NHOR}^{39}$, $-\text{C}(=\text{O})\text{NHNHR}^{39}\text{R}^{40}$, or $-\text{OCH}_2\text{CO}_2\text{H}$;

20 R^{39} , R^{40} , and R^{41} are each independently selected at each occurrence from the group: a direct bond, H, and $(\text{C}_1\text{-C}_6)\text{alkyl}$.

25

In a preferred aspect of the tenth embodiment the present invention provides a method wherein:

C_h is selected from the group: $-\text{R}^{42}\text{N}=\text{N}^+=$, $-\text{R}^{42}\text{R}^{43}\text{N}=\text{N}=$, $-\text{R}^{42}\text{N}=\text{N}$, and $-\text{R}^{42}\text{N}=\text{N}(\text{H})-$, wherein

30 $\text{R}^{42}\text{N}=\text{N}$, and $-\text{R}^{42}\text{N}=\text{N}(\text{H})-$, wherein

R^{42} is a direct bond, $(\text{C}_1\text{-C}_{10})\text{alkyl}$ substituted with 0-3

R^{44} , aryl substituted with 0-3 R^{44} , cycloalkyl

substituted with 0-3 R^{44} , heterocycle substituted with

0-3 R^{44} , heterocycloalkyl substituted with 0-3 R^{44} ,

35 aralkyl substituted with 0-3 R^{44} , or alkaryl

substituted with 0-3 R^{44} ;

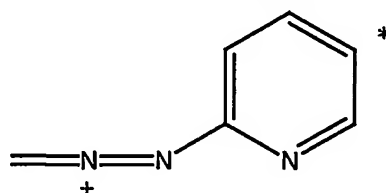
R^{43} is hydrogen, aryl substituted with 0-3 R^{44} , (C_1 - C_{10})alkyl substituted with 0-3 R^{44} , and a heterocycle substituted with 0-3 R^{44} ;

R^{44} is a direct bond, =O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{45}$,
 5 $-C(=O)R^{45}$, $-C(=O)N(R^{45})_2$, $-CHO$, $-CH_2OR^{45}$, $-OC(=O)R^{45}$, $-OC(=O)OR^{46}$, $-OR^{45}$, $-OC(=O)N(R^{45})_2$, $-NR^{45}C(=O)R^{45}$, $-NR^{47}C(=O)OR^{46}$, $-NR^{45}C(=O)N(R^{45})_2$, $-NR^{45}SO_2N(R^{45})_2$, $-NR^{47}SO_2R^{46}$, $-SO_3H$, $-SO_2R^{46}$, $-SR^{45}$, $-S(=O)R^{46}$, $-SO_2N(R^{45})_2$, $-N(R^{45})_2$, $-NHC(=NH)NHR^{45}$, $-C(=NH)NHR^{45}$,
 10 $=NOR^{45}$, NO_2 , $-C(=O)NHR^{45}$, $-C(=O)NHN(R^{45})R^{46}$, or $-OCH_2CO_2H$;

R^{45} , R^{46} , and R^{47} are each independently selected at each occurrence from the group: a direct bond, H, and (C_1 - C_6)alkyl.

15

In a preferred aspect of the tenth embodiment the present invention provides a method wherein C_h is



20 and is attached to L_n at the carbon designated with a *.

In a preferred aspect of the tenth embodiment the present invention provides a method wherein M_t is technetium-99m.

25

In a preferred aspect of the tenth embodiment the present invention provides a method wherein M_t is rhenium-186.

30 In a preferred aspect of the tenth embodiment the present invention provides a method wherein M_t is rhenium-188.

In a preferred aspect of the tenth embodiment the present invention provides a method wherein A_{L1} is a halide, a dioxygen ligand, or a functionalized aminocarboxylate.

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In a preferred aspect of the tenth embodiment the present invention provides a method wherein A_{L1} is tricine.

In a preferred aspect of the tenth embodiment the present invention provides a method wherein:

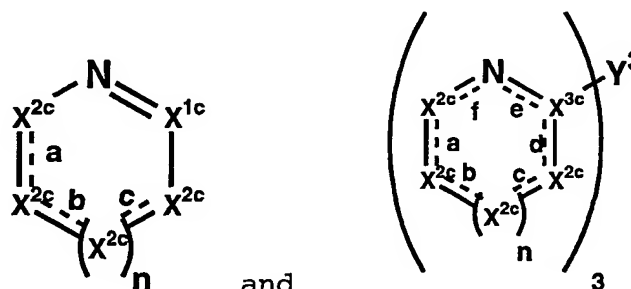
A_{L2} is selected from the group: $-A^{31}$ and $-A^{32}-W-A^{33}$;
 A^{31} is $-PR^{91}R^{92}R^{93}$ or $-AsR^{91}R^{92}R^{93}$;
 A^{32} and A^{33} are each independently $-PR^{91}R^{92}$ or $-AsR^{91}R^{92}$;
 W is a spacer group selected from the group: (C_1-C_{10}) alkyl substituted with 0-3 R^{94} , aryl substituted with 0-3 R^{94} , cycloalkyl substituted with 0-3 R^{94} , heterocycle substituted with 0-3 R^{94} , heterocycloalkyl substituted with 0-3 R^{94} , aralkyl substituted with 0-3 R^{94} and alkaryl substituted with 0-3 R^{94} ;

R^{91} , R^{92} , and R^{93} are independently selected at each occurrence from the group: (C_1-C_{10}) alkyl substituted with 0-3 R^{94} , aryl substituted with 0-3 R^{94} , cycloalkyl substituted with 0-3 R^{94} , heterocycle substituted with 0-3 R^{94} , aralkyl substituted with 0-3 R^{94} , alkaryl substituted with 0-3 R^{94} , and arylalkaryl substituted with 0-3 R^{94} ;

R^{94} is independently selected at each occurrence from the group: F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{95}$, $-C(=O)R^{95}$, $-C(=C)N(R^{95})_2$, $-CH_2OR^{95}$, $-OC(=O)R^{95}$, $-OC(=O)OR^{96}$, $-OR^{95}$, $-OC(=O)N(R^{95})_2$, $-NR^{95}C(=O)R^{95}$, $-NR^{95}C(=O)OR^{95}$, $-NR^{95}C(=O)N(R^{95})_2$, SO_3^- , $-NR^{95}SO_2N(R^{95})_2$, $-NR^{95}SO_2R^{96}$, $-SO_3H$, $-SO_2R^{95}$, $-S(=O)R^{95}$, $-SO_2N(R^{95})_2$, $-N(R^{95})_2$, $-N(R^{95})_3^+$, $-NHC(=NH)NHR^{95}$, $-C(=NH)NHR^{95}$, $=NOR^{95}$, $-NO_2$, $-C(=O)NHOR^{95}$, $-C(=O)NHN(R^{95})R^{96}$, and $-OCH_2CO_2H$; and

R^{95} and R^{96} are independently selected at each occurrence from the group: hydrogen and (C_1-C_6) alkyl.

In a preferred aspect of the tenth embodiment the present invention provides a method wherein A_{L2} is an ancillary ligand selected from the group:



5

and

wherein

n is 0 or 1;

X^{1c} is independently selected at each occurrence from the group: CR^{84} and N;

10 X^{2c} is independently selected at each occurrence from the group: CR^{84} , $CR^{84}R^{84}$, N, NR^{84} , O and S;

X^{3c} is independently selected at each occurrence from the group: C, CR^{84} , and N;

15 provided the total number of heteroatoms in each ring of the ligand A_{L2} is 1 to 4;

Y^3 is selected from the group: BR^{84-} , CR^{84} , $(P=O)$, $(P=S)$; and a, b, c, d, e and f indicate the positions of optional double bonds, provided that one of e and f is a double bond;

20 R^{64} is independently selected at each occurrence from the group:

H, (C_1-C_{10}) alkyl substituted with 0-3 R^{85} , (C_2-C_{10}) alkenyl substituted with 0-3 R^{85} , (C_2-C_{10}) alkynyl substituted with 0-3 R^{85} , aryl

25 substituted with 0-3 R^{85} , carbocycle substituted with 0-3 R^{85} , and R^{85} ;

or, alternatively, two R^{84} may be taken together with the atom or atoms to which they are attached to form a fused aromatic, carbocyclic or
30 heterocyclic ring, substituted with 0-3 R^{85} ;

R^{85} is independently selected at each occurrence from the group: =O, F, Cl, Br, I, $-CF_3$, $-CN$, $-NO_2$,

$-\text{CO}_2\text{R}^{86}$, $-\text{C}(=\text{O})\text{R}^{86}$, $-\text{C}(=\text{O})\text{N}(\text{R}^{86})_2$, $-\text{N}(\text{R}^{86})_3^+$
 $-\text{CH}_2\text{OR}^{86}$, $-\text{OC}(=\text{O})\text{R}^{86}$, $-\text{OC}(=\text{O})\text{OR}^{86a}$, $-\text{OR}^{86}$,
 $-\text{OC}(=\text{O})\text{N}(\text{R}^{86})_2$, $-\text{NR}^{86}\text{C}(=\text{O})\text{R}^{86}$, $-\text{NR}^{87}\text{C}(=\text{O})\text{OR}^{86a}$,
 $-\text{NR}^{86}\text{C}(=\text{O})\text{N}(\text{R}^{86})_2$, $-\text{NR}^{87}\text{SO}_2\text{N}(\text{R}^{86})_2$, $-\text{NR}^{87}\text{SO}_2\text{R}^{86a}$,
5 $-\text{SO}_3\text{H}$, $-\text{SO}_2\text{R}^{86a}$, $-\text{SO}_2\text{N}(\text{R}^{86})_2$, $-\text{N}(\text{R}^{86})_2$, $-\text{OCH}_2\text{CO}_2\text{H}$;
and
 R^{86} , R^{86a} , and R^{87} are each independently selected at
each occurrence from the group: hydrogen and $(\text{C}_1$ -
 $\text{C}_6)$ alkyl.

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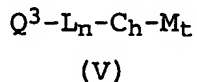
In a preferred aspect of the tenth embodiment the
present invention provides a method wherein $\text{A}_{\text{L}2}$ is
 $-\text{PR}^{48}\text{R}^{49}\text{R}^{50}$.

15 In a preferred aspect of the tenth embodiment the
present invention provides a method wherein R^{48} , R^{49} , and
 R^{50} are each aryl substituted with one R^{51} substituent.

20 In a preferred aspect of the tenth embodiment the
present invention provides a method wherein each aryl is
phenyl.

25 In a preferred aspect of the tenth embodiment the
present invention provides a method wherein each R^{51}
substituent is SO_3H or SO_3^- , in the meta position.

30 In a preferred aspect of the tenth embodiment the
present invention provides a method wherein the
radiopharmaceutical is a compound of Formula V:



wherein

35 Q^3 is an inhibitor of APP processing and/or the production
of beta-amyloid production;
 L_n is a linking group of the formula
 $-\text{M}^{31}-\text{Y}^{31}(\text{CR}^{31}\text{R}^{32})_f(\text{Z}^1)_f\text{Y}^{32}-\text{M}^{32}-$;

wherein

M^{31} is $-(CH_2)_g Z^{31}]_{g'}-(CR^{31}R^{32})_{g''}-$;

M^{32} is $-(CR^{31}R^{32})_{g''}-[Z^{31}(CH_2)_g]_{g'}-$;

g is independently 0-10;

5 g' is independently 0-1;

g'' is independently 0-10;

f is independently 0-10;

f' is independently 0-10;

f'' is independently 0-1;

10 Y^{31} and Y^{32} , at each occurrence, are independently selected from: a direct bond, $-O-$, $-NR^{32}-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)O-$, $-C(=O)NH-$, $-C(=NR^{32})-$, $-S-$, $-SO-$, $-SO_2-$, $-SO_3-$, $-NHC(=O)-$, $-(NH)_2C(=O)-$, $-(NH)_2C=S-$;

Z^{31} is independently selected at each occurrence from a (C_6 -
15 C_{14}) saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{33} ; and a heterocyclic ring system, optionally substituted with 0-4 R^{33} ;

R^{31} and R^{32} are independently selected at each occurrence
20 from: hydrogen; (C_1-C_{10}) alkyl substituted with 0-5 R^{33} ; alkaryl wherein the aryl is substituted with 0-5 R^{33} ;

R^{33} is independently selected at each occurrence from the group: hydrogen, $-OH$, $-NHR^{34}$, $-C(=O)R^{34}$, $-OC(=O)R^{34}$, $-OC(=O)OR^{34}$, $-C(=O)OR^{34}$, $-C(=O)NR^{34}$, $-CN$, $-SR^{34}$, $-SOR^{34}$,
25 $-SO_2R^{34}$, $-NHC(=O)R^{34}$, $-NHC(=O)NHR^{34}$, or $-NHC(=S)NHR^{34}$;
and

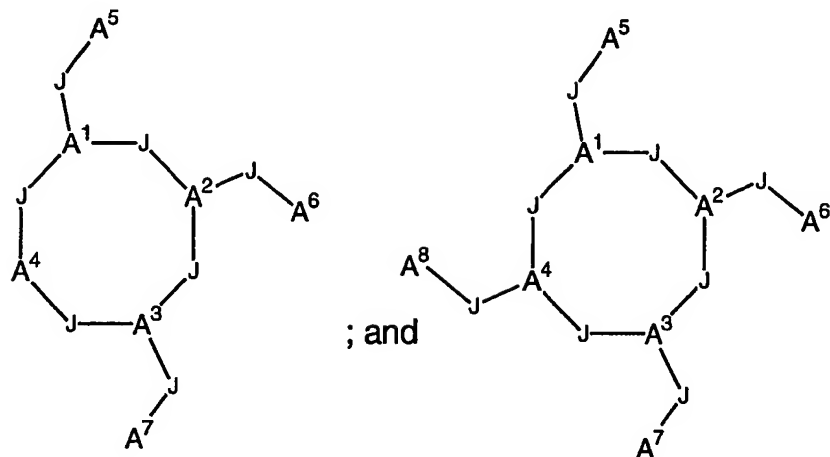
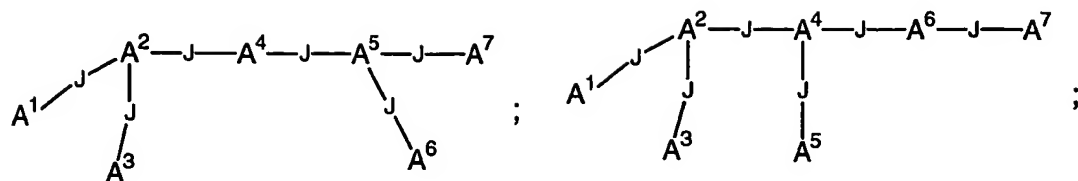
R^{34} is independently selected at each occurrence from the group: hydrogen; (C_1-C_6) alkyl; benzyl, and phenyl;

C_h is a radionuclide metal chelator coordinated to a
30 transition metal radionuclide M_t ;

M_t is a transition metal radionuclide;

and pharmaceutically acceptable salts thereof.

In a preferred aspect of the tenth embodiment the
35 present invention provides a method wherein C_h is selected from the group:



wherein:

A¹, A², A³, A⁴, A⁵, A⁶, and A⁷ are independently

5 selected at each occurrence from the group:

NR⁶⁰R⁶¹, S, SH, S(Pg), O, OH, PR⁶²R⁶³, P(O)R⁶²R⁷³,
P(S)R⁶²R⁶³, P(NR⁶⁷)R⁶²R⁶³;

J is a direct bond, CH, or a spacer group selected

10 from the group: (C₁-C₁₀)alkyl substituted with 0-3 R⁷², aryl substituted with 0-3 R⁷², cycloalkyl substituted with 0-3 R⁷², heterocycloalkyl substituted with 0-3 R⁷², aralkyl substituted with 0-3 R⁷² and alkaryl substituted with 0-3 R⁷²;

15 R⁶⁰, R⁶¹, R⁶², R⁶³, and R⁶⁴ are each independently selected from the group: a direct bond, hydrogen, (C₁-C₁₀)alkyl substituted with 0-3 R⁷², aryl substituted with 0-3 R⁷², cycloalkyl substituted with 0-3 R⁷², heterocycloalkyl substituted with
20 0-3 R⁷², aralkyl substituted with 0-3 R⁷², alkaryl

- substituted with 0-3 R⁷² substituted with 0-3 R⁷²
 and an electron, provided that when one of R⁷⁰ or
 R⁷¹ is an electron, then the other is also an
 electron, and provided that when one of R⁷² or R⁷³
 is an electron, then the other is also an
 electron;
 additionally, R⁶⁰ and R⁶¹ may combine to form =C(C₁-
 C₃)alkyl (C₁-C₃)alkyl;
 R⁷² is independently selected at each occurrence from
 the group: a direct bond, =O, F, Cl, Br, I, -CF₃,
 -CN, -CO₂R⁷³, -C(=O)R⁷³, -C(=O)N(R⁷³)₂, -CHO,
 -CH₂OR⁷³, -OC(=O)R⁷³, -OC(=O)OR^{73a}, -OR⁷³,
 -OC(=O)N(R⁷³)₂, -NR⁷³C(=O)R⁷³, -NR⁷⁴C(=O)OR^{73a},
 -NR⁷³C(=O)N(R⁷³)₂, -NR⁷⁴SO₂N(R⁷³)₂, -NR⁷⁴SO₂R^{73a},
 -SO₃H, -SO₂R^{73a}, -SR⁷³, -S(=O)R^{73a}, -SO₂N(R⁷³)₂,
 -N(R⁷³)₂, -NHC(=NH)NHR⁷³, -C(=NH)NHR⁷³, =NOR⁷³,
 NO₂, -C(=O)NHR⁷³, -C(=O)NHN(R⁷³)R^{73a}, -OCH₂CO₂H,
 2-(1-morpholino)ethoxy,
 (C₁-C₅)alkyl, (C₂-C₄)alkenyl, (C₃-C₆)cycloalkyl, (C₃-
 C₆)cycloalkylmethyl, (C₂-C₆)alkoxyalkyl,
 aryl substituted with 0-2 R⁷³,
 a 5-10-membered heterocyclic ring system containing 1-
 4 heteroatoms independently selected from N, S,
 and O;
 R⁷³, R^{73a}, and R⁷⁴ are independently selected at each
 occurrence from the group: a direct bond, (C₁-
 C₆)alkyl, phenyl, benzyl, (C₁-C₆)alkoxy, halide,
 nitro, cyano, and trifluoromethyl; and
 Pg is a thiol protecting group capable of being
 displaced upon reaction with a radionuclide.

- In a preferred aspect of the tenth embodiment the
 present invention provides a method wherein C_h is selected
 from the group:
 diethylenetriamine- pentaacetic acid (DTPA);
 ethylenediamine-tetraacetic acid (EDTA); 1,4,7,10-
 tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid
 (DOTA);

1,4,7,10-tetraaza-cyclododecane-N,N',N''-triacetic acid;
 hydroxybenzyl-ethylene-diamine diacetic acid;
 N,N'-bis(pyridoxyl- 5-phosphate)ethylene diamine;
 N,N'-diacetate, 3,6,9-triaza-12- oxa-3,6,9-
 5 tricarboxymethylene-10-carboxy-13-phenyl-tridecanoic acid;
 1,4,7-triazacyclononane-N,N',N''-triacetic acid;
 1,4,8,11- tetraazacyclo-tetradecane-N,N'N'',N''''-
 tetraacetic acid;
 2,3-bis(S- benzoyl)mercaptoacetamido-propanoic acid.

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In a preferred aspect of the tenth embodiment the present invention provides a method wherein M_t is indium-111 or gallium-68.

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In a preferred aspect of the tenth embodiment the present invention provides a method wherein the neurological disease is Alzheimer's disease.

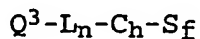
20 In an eleventh embodiment the present invention provides a method for diagnosing a neurological disease involving APP processing and/or the production of beta-amyloid production within a mammalian body comprising:

(a) administering a diagnostically effective amount of an ultrasound contrast agent composition inhibitor of APP
 25 processing and/or the production of beta-amyloid production; and

(b) imaging the area of the patient wherein the disease is located.

30 In a preferred aspect of the eleventh embodiment the present invention provides a method for diagnosing a neurological disease wherein the an ultrasound contrast agent composition is of the formula

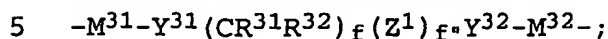
35



wherein

Q^3 is an inhibitor of APP processing and/or the production of beta-amyloid production;

L_n is a linking group of the formula



wherein

M^{31} is $-(CH_2)_gZ^{31}]_{g'}-(CR^{31}R^{32})_{g''}-$;

M^{32} is $-(CR^{31}R^{32})_{g''}-[Z^{31}(CH_2)_g]_{g'}-$;

g is independently 0-10;

10 g' is independently 0-1;

g'' is independently 0-10;

f is independently 0-10;

f' is independently 0-10;

f'' is independently 0-1;

15 Y^{31} and Y^{32} , at each occurrence, are independently selected from: a direct bond, $-O-$, $-NR^{32}-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)O-$, $-C(=O)NH-$, $-C(=NR^{32})-$, $-S-$, $-SO-$, $-SO_2-$, $-SO_3-$, $-NHC(=O)-$, $-(NH)_2C(=O)-$, $-(NH)_2C=S-$;

20 Z^{31} is independently selected at each occurrence from a (C_6-C_{14}) saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{33} ; and a heterocyclic ring system, optionally substituted with 0-4 R^{33} ;

25 R^{31} and R^{32} are independently selected at each occurrence from: hydrogen; (C_1-C_{10})alkyl substituted with 0-5 R^{33} ; alkaryl wherein the aryl is substituted with 0-5 R^{33} ;

30 R^{33} is independently selected at each occurrence from the group: hydrogen, $-OH$, $-NHR^{34}$, $-C(=O)R^{34}$, $-OC(=O)R^{34}$, $-OC(=O)OR^{34}$, $-C(=O)OR^{34}$, $-C(=O)NR^{34}$, $-CN$, $-SR^{34}$, $-SOR^{34}$, $-SO_2R^{34}$, $-NHC(=O)R^{34}$, $-NHC(=O)NHR^{34}$, or $-NHC(=S)NHR^{34}$; and

R^{34} is independently selected at each occurrence from the group: hydrogen; (C_1-C_6)alkyl; benzyl, and phenyl;

S_f is a surfactant which is a lipid or a compound of the

35

formula: $A^9E^1A^{10}$;

A^9 is selected from the group: OH and OR^{27} ;

A¹⁰ is OR²⁷;

R²⁷ is C(=O)C₁₋₂₀ alkyl;

E¹ is C₁₋₁₀ alkylene substituted with 1-3 R²⁸;

5 R²⁸ is independently selected at each occurrence from the group: R³⁰, -PO₃H-R³⁰, =O, -CO₂R²⁹, -C(=O)R²⁹, -C(=O)N(R²⁹)₂, -CH₂OR²⁹, -OR²⁹, -N(R²⁹)₂, C₁-C₅ alkyl, and C₂-C₄ alkenyl;

R²⁹ is independently selected at each occurrence from the group: R³⁰, H, C₁-C₆ alkyl, phenyl, benzyl, and

10 trifluoromethyl;

R³⁰ is a bond to L_n;

and a pharmaceutically acceptable salt thereof.

15 In a preferred aspect of the eleventh embodiment the present invention provides a method wherein the ultrasound contrast agent composition further comprises: 1,2-dipalmitoyl-sn-glycero-3-phosphotidic acid, 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine, and N-
20 (methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine.

In a preferred aspect of the eleventh embodiment the present invention provides a method wherein the ultrasound
25 contrast agent composition further comprises an echogenic gas.

In a preferred aspect of the eleventh embodiment the present invention provides a method wherein the echogenic
30 gas is C₂₋₅ perfluorocarbon.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in
35 combination in a single embodiment. Conversely, various features of the invention which are for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

DEFINITIONS

As used herein, the term "A β " denotes the protein designated A β , β -amyloid peptide, and sometimes β /A4, in the art. A β is an approximately 4.2 kilodalton (kD) protein of about 39 to 43 amino acids found in amyloid plaques, the walls of meningeal and parenchymal arterioles, small arteries, capillaries, and sometimes, venules. The isolation and sequence data for the first 28 amino acids are described in U.S. Pat. No 4,666,829. The 43 amino acid sequence is:

```

1
Asp  Ala  Glu  Phe  Arg  His  Asp  Ser  Gly  Tyr
11
Glu  Val  His  His  Gln  Lys  Leu  Val  Phe  Phe
21
Ala  Glu  Asp  Val  Gly  Ser  Asn  Lys  Gly  Ala
31
Ile  Ile  Gly  Leu  Met  Val  Gly  Gly  Val  Val
41
Ile  Ala  Thr.

```

However, a skilled artisan knows that fragments generated by enzymatic degradation can result in loss of amino acids 1-10 and/or amino acids 39-43. Thus, amino acid sequence 1-43 represents the maximum sequence of amino acids for A β peptide.

The term "APP", as used herein, refers to the protein known in the art as β amyloid precursor protein. This protein is the precursor for A β and through the activity of "secretase" enzymes, as used herein, it is processed into A β . Differing secretase enzymes, known in the art, have been designated β secretase, generating the N-terminus of A β , α secretase cleaving around the 16/17 peptide bond in A β , and " γ secretases", as used herein, generating C-terminal A β fragments ending at position 38, 39, 40, 41, 42, and 43 or generating C-terminal extended precursors which are subsequently truncated to the above polypeptides.

The compounds herein described may have asymmetric centers. Compounds of the present invention containing an asymmetrically substituted atom may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis from optically active starting materials. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. Cis and trans geometric isomers of the compounds of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms. All chiral, diastereomeric, racemic forms and all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomeric form is specifically indicated.

The term "substituted," as used herein, means that any one or more hydrogens on the designated atom is replaced with a selection from the indicated group, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound. When a substituent is keto (i.e., =O), then 2 hydrogens on the atom are replaced.

When any variable (e.g., R^{5b}) occurs more than one time in any constituent or formula for a compound, its definition at each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 0-2 R^{5b} , then said group may optionally be substituted with up to two R^{5b} groups and R^{5b} at each occurrence is selected independently from the definition of R^{5b} . Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

When a bond to a substituent is shown to cross a bond connecting two atoms in a ring, then such substituent may be bonded to any atom on the ring. When a substituent is listed without indicating the atom via which such substituent is bonded to the rest of the compound of a

given formula, then such substituent may be bonded via any atom in such substituent. Combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

5 As used herein, "alkyl" or "alkylene" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms; for example, "C₁-C₆ alkyl" denotes alkyl having 1 to 6 carbon atoms. Examples of alkyl include, but
10 are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, t-butyl, pentyl, and hexyl. Preferred "alkyl" group, unless otherwise specified, is "C₁-C₄ alkyl".

 As used herein, "alkenyl" or "alkenylene" is intended
15 to include hydrocarbon chains of either a straight or branched configuration and one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain. Examples of "C₂-C₆ alkenyl" include, but
20 are not limited to, ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 3-methyl-2-butenyl, 2-pentenyl, 3-pentenyl, hexenyl, and the like.

 As used herein, "alkynyl" or "alkynylene" is intended to include hydrocarbon chains of either a straight or branched configuration and one or more carbon-carbon triple
25 bonds which may occur in any stable point along the chain, such as ethynyl, 1-propynyl, 2-propynyl, 1-butylnyl, 2-butylnyl, 3-butylnyl, and the like.

 "Alkoxy" or "alkyloxy" represents an alkyl group as defined above with the indicated number of carbon atoms
30 attached through an oxygen bridge. Examples of alkoxy include, but are not limited to, methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, t-butoxy, n-pentoxy, and s-pentoxy. Preferred alkoxy groups are methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy,
35 t-butoxy. Similarly, "alkylthio" or "thioalkoxy" is represents an alkyl group as defined above with the indicated number of carbon atoms attached through a sulphur bridge.

"Halo" or "halogen" as used herein refers to fluoro, chloro, bromo, and iodo. Unless otherwise specified, preferred halo is fluoro and chloro. "Counterion" is used to represent a small, negatively charged species such as chloride, bromide, hydroxide, acetate, sulfate, and the like.

"Haloalkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms, substituted with 1 or more halogen (for example $-C_vF_w$ where $v = 1$ to 3 and $w = 1$ to $(2v+1)$). Examples of haloalkyl include, but are not limited to, trifluoromethyl, trichloromethyl, pentafluoroethyl, pentachloroethyl, 2,2,2-trifluoroethyl, 2,2-difluoroethyl, heptafluoropropyl, and heptachloropropyl. "Haloalkoxy" is intended to mean a haloalkyl group as defined above with the indicated number of carbon atoms attached through an oxygen bridge; for example trifluoromethoxy, pentafluoroethoxy, 2,2,2-trifluoroethoxy, and the like. "Halothioalkoxy" is intended to mean a haloalkyl group as defined above with the indicated number of carbon atoms attached through a sulphur bridge.

"Cycloalkyl" is intended to include saturated ring groups, having the specified number of carbon atoms. For example, "C₃-C₆ cycloalkyl" denotes such as cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

As used herein, "carbocycle" is intended to mean any stable 3- to 7-membered monocyclic or bicyclic or 7- to 13-membered bicyclic or tricyclic, any of which may be saturated, partially unsaturated, or aromatic. Examples of such carbocycles include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, cyclooctyl, [3.3.0]bicyclooctane, [4.3.0]bicyclononane, [4.4.0]bicyclodecane (decalin), [2.2.2]bicyclooctane, fluorenyl, phenyl, naphthyl, indanyl, adamantyl, or tetrahydronaphthyl (tetralin). Preferred "carbocycle" are cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

As used herein, the term "heterocycle" or "heterocyclic ring" is intended to mean a stable 5- to 7-membered monocyclic or bicyclic or 7- to 14-membered bicyclic heterocyclic ring which is saturated partially unsaturated or unsaturated (aromatic), and which consists of carbon atoms and 1, 2, 3 or 4 heteroatoms, preferably 1, 2, or 3 heteroatoms, independently selected from the group consisting of N, O and S and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The nitrogen and sulfur heteroatoms may optionally be oxidized. The heterocyclic ring may be attached to its pendant group at any heteroatom or carbon atom which results in a stable structure. The heterocyclic rings described herein may be substituted on carbon or on a nitrogen atom if the resulting compound is stable. If specifically noted, a nitrogen in the heterocycle may optionally be quaternized. It is preferred that when the total number of S and O atoms in the heterocycle exceeds 1, then these heteroatoms are not adjacent to one another. It is preferred that the total number of S and O atoms in the heterocycle is not more than 1.

Examples of heterocycles include, but are not limited to, 1H-indazole, 2-pyrrolidinyl, 2H,6H-1,5,2-dithiazinyl, 2H-pyrrolyl, 3H-indolyl, 4-piperidinyl, 4aH-carbazole, 4H-quinolizinyl, 6H-1,2,5-thiadiazinyl, acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazalonyl, carbazolyl, 4aH-carbazolyl, b-carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2H,6H-1,5,2-dithiazinyl, dihydrofuro[2,3-b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolyl, imidazolyl, 1H-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl,

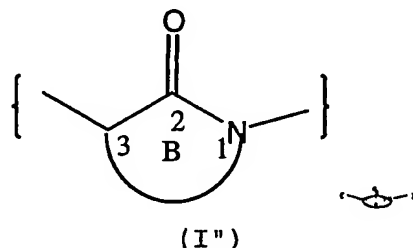
oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl,
1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl,
oxazolyl, oxazolidinylperimidinyl, phenanthridinyl,
phenanthrolinyl, phenarsazinyl, phenazinyl, phenothiazinyl,
5 phenoxathiinyl, phenoxazinyl, phthalazinyl, piperazinyl,
piperidinyl, pteridinyl, piperidonyl, 4-piperidonyl,
pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl,
pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole,
pyridoimidazole, pyridothiazole, pyridinyl, pyridyl,
10 pyrimidinyl, pyrrolidinyl, pyrrolinyl, pyrrolyl,
quinazolinyl, quinolinyl, 4H-quinoliziny, quinoxaliny,
quinuclidinyl, carbolinyl, tetrahydrofuranyl,
tetrahydroisoquinolinyl, tetrahydroquinolinyl,
6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl,
15 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl,
thianthrenyl, thiazolyl, thienyl, thienothiazolyl,
thienooxazolyl, thienoimidazolyl, thiophenyl, triazinyl,
1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl,
1,3,4-triazolyl, xanthenyl. Preferred 5 to 10 membered
20 heterocycles include, but are not limited to, pyridinyl,
pyrimidinyl, triazinyl, furanyl, thienyl, thiazolyl,
pyrrolyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl,
tetrazolyl, benzofuranyl, benzothiofuranyl, indolyl,
benzimidazolyl, 1H-indazolyl, oxazolidinyl, isoxazolidinyl,
25 benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl,
quinolinyl, and isoquinolinyl. Preferred 5 to 6 membered
heterocycles include, but are not limited to, pyridinyl,
pyrimidinyl, triazinyl, furanyl, thienyl, thiazolyl,
pyrrolyl, piperazinyl, piperidinyl, pyrazolyl, imidazolyl,
30 oxazolyl, isoxazolyl, tetrazolyl; more preferred 5 to 6
membered heterocycles include, but are not limited to,
pyridinyl, pyrimidinyl, triazinyl, furanyl, thienyl,
thiazolyl, piperazinyl, piperidinyl, pyrazolyl, imidazolyl,
and tetrazolyl. Also included are fused ring and spiro
35 compounds containing, for example, the above heterocycles.

As used herein, the term "aryl", "C₆-C₁₀ aryl" or aromatic residue, is intended to mean an aromatic moiety containing the specified number of carbon atoms; for

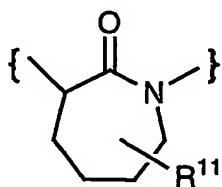
example phenyl, pyridinyl or naphthyl. Unless otherwise specified, "aryl" may be unsubstituted or substituted with 0 to 3 groups selected from H, -OH, -OCH₃, Cl, F, Br, I, CN, -NO₂, -NH₂, -N(CH₃)H, -N(CH₃)₂, -CF₃, -OCF₃, -C(=O)CH₃,
 5 -SCH₃, -S(=O)CH₃, -S(=O)₂CH₃, -CH₃, -CH₂CH₃, -CO₂H, and -CO₂CH₃.

The phrase "additional lactam carbons", as used herein, is intended to denote the number of optional carbon atoms in the lactam ring B of Formula (I). Formula (I"):

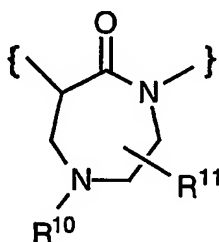
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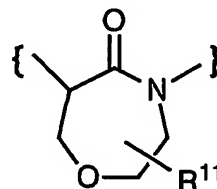
represents the lactam ring B of Formula (I). Additional lactam carbons are carbons in lactam ring B other than the carbons numbered 2 and 3 in the backbone of the formula.
 15 The additional lactam carbons may be optionally replaced by a heteroatom selected from oxygen, nitrogen and sulfur. Lactam ring B contains 1, 2, 3, 4, 5, 6 or 7 optional carbons, wherein one optional carbon may optionally be
 20 replaced by a heteroatom, such that the total number of members of lactam ring B, including atoms numbered 1, 2 and 3 in the backbone, does not exceed 10. It is preferred that the total number of atoms of lactam ring B is 6, 7 or 8; it is more preferred that the total number of atoms of
 25 lactam ring B is seven. Examples of lactam ring B include:



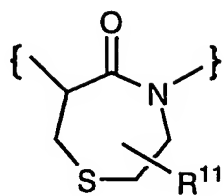
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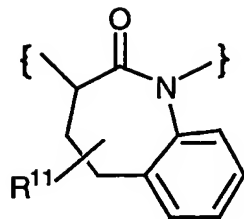
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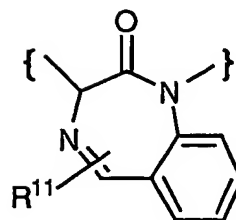
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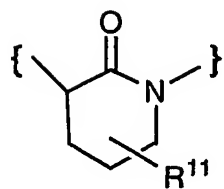
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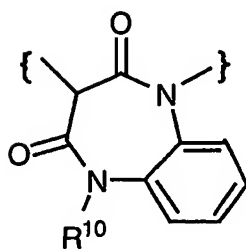
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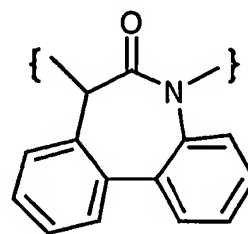
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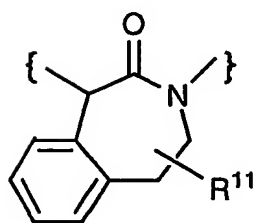
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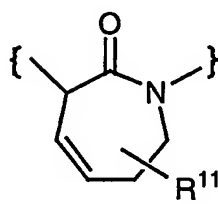
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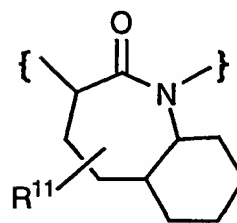
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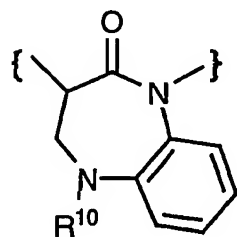
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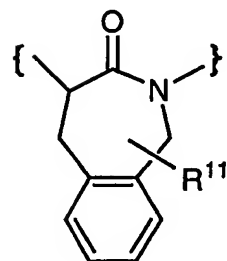
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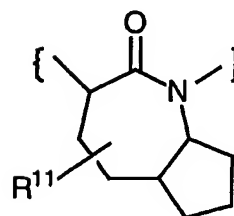
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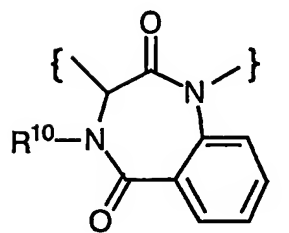
B13



B14



B15



B16

but are not intended to limit the invention. Preferred examples of lactam ring B are B1, B2, B5, B6, B8, B9, B13, and B16; more preferred examples of lactam ring B are B1, B6, B8, B9, and B13. Even more preferred examples of lactam ring B are B1 and B6. Preferred examples of substituent R¹⁰ or R¹¹ on lactam B are methyl, ethyl, phenyl, 4-fluorophenyl, 4-chlorophenyl, 4-trifluorophenyl, (4-fluorophenyl)methyl, (4-chlorophenyl)methyl, and (4-trifluorophenyl)methyl.

As used herein, "macromolecule" or "complex of macromolecules", is intended to mean a cellular component involved directly or indirectly in APP processing and the production of A β peptide. By indirectly, its effect on APP processing may be mediated by intervening molecules. An example of a "macromolecule" or "complex of macromolecules" is presenilin 1 or endogenous cleavage N terminal or C terminal fragments of presenilin 1. Additional examples of a "macromolecule" or "complex of macromolecules" is presenilin 2, a homolog of presenilin 1 or a homolog of presenilin 2.

It is envisaged that the scope of "macromolecule" or "complex of macromolecules" involved in APP processing can be found in a wide variety of sources. Sources of a "macromolecule" or "complex of macromolecules" are considered to be materials suspected or known to contain a macromolecule involved in APP processing. Examples of a material suspected or known to contain a macromolecule involved in APP processing include, but are not limited to, purified proteins; suspensions of proteins; cells, tissues or organs, derived from prokaryotes or eucaryotes; and macromolecules derived from recombinant expression systems. Examples of cells include, but are not limited to, HEK293 cells, IMR 32 cells, RAJI cells, CHO cells, U-937 cells, and THP-1 cells; preferably HEK293 or TNP-1 cells. Examples of tissues or organs include, but are not limited to, spleen, brain, and testes. Examples of prokaryotes include, but are not limited to, bacteria, more preferably E. coli. Examples of eucaryotes include, but are not

limited to, mouse, rat, guinea pig, bovine, porcine, monkey, human, and nematodes (preferably *C. elegans*). An example of a suspension of protein includes, but is not limited to, lipid systems. An example of a macromolecule
5 derived from recombinant expression systems includes, but is not limited to, *C. elegans* knockout of Sel-12 and reintroduction of PS-1. (See Levitan, D. and Greenwald, I., *Nature*, 377, pp351-354, 1995.)

It is understood that one skilled in the art can
10 readily determine the scope of the term "binding site" and "specific binding site" as used herein. For further guidance, the tagged compounds of the present invention, for example (I-7T), (I-11T), and (I-43T), bind to a specific site on one or more macromolecules involved
15 directly or indirectly in APP processing and the production of A β peptide, and thus effect a decrease in the production of A β peptide. One skilled in the art can readily determine whether other compounds, which are inhibitors of beta-amyloid production, bind to a same site as the tagged
20 compounds of the present invention by using the assays disclosed herein. However, it is understood that within the scope of the present disclosure the phrase "is identified by a compound of Formula (I-#)" or "is a specific binding site for a compound of Formula (I-#)"
25 refers to defining the physical site on the macromolecule wherein a compound of Formula (I-#) binds to and not to a molecular reaction of binding. Thus, the phrase "a specific binding site for a compound of Formula (I-#)" does not require the compound of Formula (I-#) to be present.

30 As used herein, "potential inhibitor of beta-amyloid production" is intended to mean any compound which is being screened for activity to inhibit the production of beta-amyloid peptide, or the proteolytic activity leading to the production of beta-amyloid peptide, using the assay of the
35 invention described herein. It is understood that a "potential inhibitor of beta-amyloid production", which is active in the assay of the invention for inhibiting the production of beta-amyloid peptide, can subsequently be

used in the assay of the invention as a "beta-amyloid peptide inhibitor", as defined below, once the compound has been tagged. It is also understood that a "potential inhibitor of beta-amyloid production", which is active in the assay of the invention for inhibiting the production of beta-amyloid peptide, can subsequently be used in pharmaceutical compositions for the treatment of degenerative neurological disorders involving beta-amyloid production, preferably for the treatment of Alzheimer's disease.

As used herein, "beta-amyloid peptide inhibitor" or "inhibitor of beta-amyloid production" is intended to mean any compound which inhibits the production of beta-amyloid peptide, or the proteolytic activity leading to the production of beta-amyloid peptide. Examples of a beta-amyloid peptide inhibitor include, but are not limited to, the scope of compounds of Formula (I), examples of which are disclosed herein. However, it is contemplated for use in the invention that compounds beyond the scope of compounds of Formula (I) may be used in the invention. Additional examples of a beta-amyloid peptide inhibitor, contemplated by the invention, include, but are not limited to, 5-amino-6-cyclohexyl-4-hydroxy-hexanamide derivatives disclosed in United States patent US 5,703,129, issued Dec. 30, 1997; N-aryl amino acid esters and N-heteroaryl amino acid esters disclosed in PCT application WO98/22441 (published May 28, 1998; priority USSN 08/755,444); N-arylacetyl amino acid amides, N-heteroarylacetyl amino acid amides, and N-alkylacetyl amino acid amides disclosed in PCT application WO98/22433 (published May 28, 1998; priority USSN 08/807,538); N-arylacetyl amino acid esters, N-heteroarylacetyl amino acid esters, and N-alkylacetyl amino acid esters disclosed in PCT application WO98/22430 (published May 28, 1998; priority USSN 08/754,895); N-aryl amino acid derivatives and N-heteroaryl amino acid derivatives disclosed in PCT application WO98/22493 (published May 28, 1998; priority USSN 08/755,334); amino acid derivatives disclosed in PCT application WO98/22494

(published May 28, 1998; priority USSN 08/808,528, 08/807,528, 08/807,427); cycloalkyl, lactam, lactone and related compounds disclosed in PCT application WO98/28268 (published July 2, 1998, priority USSN 08/780,025); all
5 references of which are hereby incorporated by reference in their entirety.

As used herein, "tagged inhibitor of beta-amyloid production", is intended to mean "beta-amyloid peptide inhibitor" compounds which are tagged. By "tagged" or
10 "tagged inhibitor of beta-amyloid production" or "tagged compound", it is meant that the subject beta-amyloid peptide inhibitor compounds contain a tag which is suitable for detection in an assay system or upon administration to a mammal. Suitable tags are known to those skilled in the
15 art and include, for example, radioisotopes, fluorescent groups, biotin (in conjunction with streptavidin complexation), and photoaffinity groups. As used herein, "radiolabeled compound" or "radiolabeled inhibitor" refers to a tagged inhibitor of beta-amyloid production wherein
20 the tag is a radioisotope.

For purposes of in vivo diagnostic imaging, by "radiolabeled" it is meant that the subject inhibitors of beta-amyloid production contain a radioisotope which is suitable for administration to a mammalian patient.
25 Preferred radioisotopes for in vivo diagnostic imaging by positron emission tomography (PET) are ^{11}C , ^{18}F , ^{123}I , and ^{125}I .

Suitable radioisotopes are known to those skilled in the art and include, for example, isotopes of halogens
30 (such as chlorine, fluorine, bromine and iodine), and metals including technetium and indium. Preferred radioisotopes include ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{123}I , ^{125}I , ^{131}I . Radiolabeled compounds of the invention may be prepared using standard radiolabeling procedures well known
35 to those skilled in the art. Suitable synthesis methodology is described in detail below. As discussed below, the beta-amyloid peptide inhibitor compounds of the invention may be radiolabeled either directly (that is, by

incorporating the radiolabel directly into the compounds) or indirectly (that is, by incorporating the radiolabel into the compounds through a chelating agent, where the chelating agent has been incorporated into the compounds).

5 Also, the radiolabeling may be isotopic or nonisotopic. With isotopic radiolabeling, one group already present in the compounds of the invention described above is substituted with (exchanged for) the radioisotope. With nonisotopic radiolabeling, the radioisotope is added to the

10 compounds without substituting with (exchanging for) an already existing group. Direct and indirect radiolabeled compounds, as well as isotopic and nonisotopic radiolabeled compounds are included within the phrase "radiolabeled compounds" as used in connection with the present

15 invention. Such radiolabeling should also be reasonably stable, both chemically and metabolically, applying recognized standards in the art. Also, although the compounds of the invention may be labeled in a variety of fashions with a variety of different radioisotopes, as

20 those skilled in the art will recognize, such radiolabeling should be carried out in a manner such that the high binding affinity and specificity of the unlabeled or untagged inhibitor of beta-amyloid production compounds of the invention to the macromolecule involved in APP

25 processing is not significantly affected. By not significantly affected, it is meant that the binding affinity and specificity is not affected more than about 3 log units, preferably not more than about 2 log units, more preferably not more than about 1 log unit, even more

30 preferably not more than about 500%, and still even more preferably not more than about 250%, and most preferably the binding affinity and specificity is not affected at all.

Examples of a tagged inhibitor of beta-amyloid

35 production include, but are not limited to, the scope of compounds of Formula (I), examples of which are disclosed herein. However, it is contemplated that tagged compounds beyond the scope of compounds of Formula (I) may be used in

the invention. Additional examples of a tagged inhibitor of beta-amyloid production, contemplated by the invention, include, but are not limited to, beta-amyloid peptide inhibitors disclosed in US 5,703,129, issued Dec. 30, 1997;
5 WO98/22441 (published May 28, 1998); WO98/22433 (published May 28, 1998); WO98/22430 (published May 28, 1998); WO98/22493 (published May 28, 1998); WO98/22494 (published May 28, 1998); and WO98/28268 (published July 2, 1998), which inhibitors can be tagged for use in the invention.
10 Preferred examples of a tagged inhibitor of beta-amyloid production are compounds of Formula (I) and compounds of WO98/28268 (published July 2, 1998) which can be tagged. More preferred are compounds of Formula (I).

For radiolabeled compounds, the label may appear at
15 any position on the beta-amyloid peptide inhibitor. Preferred radiolabeled compounds of the invention are beta-amyloid peptide inhibitor radiolabeled with tritium. More preferred radiolabeled compounds of the invention are radiolabeled compounds wherein the radiolabel is located on
20 R³ of Formula (I).

As used herein, when the tagged inhibitor of beta-amyloid production is tagged with a photoaffinity group or photoaffinity labeled, the term "photoaffinity group" or "photoaffinity labeled" refers to a substituent on the
25 inhibitor which can be activated by photolysis at an appropriate wavelength to undergo a crosslinking photochemical reaction with the macromolecule to which it is associated. An example of a "photoaffinity group" is a benzophenone substituent.

30 In the present invention it has also been discovered that the radiolabeled compounds above are useful as inhibitors of beta-amyloid peptide production and thus the radiolabeled compounds of the invention may also be employed for therapeutic purposes, in addition to the
35 diagnostic usage described above.

As used herein, "inhibitory concentration" is intended to mean the concentration at which the "potential inhibitor of beta-amyloid production" compound screened in the assay

of the invention inhibits a measurable percentage of beta-amyloid peptide production. Examples of "inhibitory concentration" values range from IC₅₀ to IC₉₀, and are preferably, IC₅₀, IC₆₀, IC₇₀, IC₈₀, or IC₉₀, which represent
5 50%, 60%, 70%, 80% and 90% reduction in beta-amyloid peptide production, respectively. More preferably, the "inhibitory concentration" is measured as the IC₅₀ value. It is understood that an designation for IC₅₀ is the half maximal inhibitory concentration.

10 The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals
15 without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the
20 parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the
25 like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from
30 inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic,
35 phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, benzenesulfonic, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting
5 the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists
10 of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

"Stable compound" and "stable structure" are meant to
15 indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

The term "metallopharmaceutical" means a
20 pharmaceutical comprising a metal. The metal is the cause of the imageable signal in diagnostic applications and the source of the cytotoxic radiation in radiotherapeutic applications. Radiopharmaceuticals are
25 metallopharmaceuticals in which the metal is a radioisotope.

By "reagent" is meant a compound of this invention capable of direct transformation into a
metallopharmaceutical of this invention. Reagents may be utilized directly for the preparation of the
30 metallopharmaceuticals of this invention or may be a component in a kit of this invention.

The term "binding agent" means a metallopharmaceutical of this invention having affinity for and capable of
binding a binding site specific for said tagged inhibitor
35 of beta-amyloid production.

A "cyclodextrin" is a cyclic oligosaccharide. Examples of cyclodextrins include, but are not limited to, α -cyclodextrin, hydroxyethyl- α -cyclodextrin,

hydroxypropyl- α -cyclodextrin, β -cyclodextrin,
hydroxypropyl- β -cyclodextrin, carboxymethyl- β -cyclodextrin,
dihydroxypropyl- β -cyclodextrin,
hydroxyethyl- β -cyclodextrin, 2,6

5 di-O-methyl- β -cyclodextrin, sulfated- β -cyclodextrin,
 γ -cyclodextrin, hydroxypropyl- γ -cyclodextrin,
dihydroxypropyl- γ -cyclodextrin, hydroxyethyl- γ -cyclodextrin,
and sulfated γ -cyclodextrin.

As used herein, the term "polycarboxyalkyl" means an
10 alkyl group having between two and about 100 carbon atoms
and a plurality of carboxyl substituents; and the term
"polyazaalkyl" means a linear or branched alkyl group
having between two and about 100 carbon atoms, interrupted
by or substituted with a plurality of amine groups.

15 A "reducing agent" is a compound that reacts with a
radionuclide, which is typically obtained as a relatively
unreactive, high oxidation state compound, to lower its
oxidation state by transferring electron(s) to the
radionuclide, thereby making it more reactive. Reducing
20 agents useful in the preparation of radiopharmaceuticals
and in diagnostic kits useful for the preparation of said
radiopharmaceuticals include but are not limited to
stannous chloride, stannous fluoride, formamidine sulfinic
acid, ascorbic acid, cysteine, phosphines, and cuprous or
25 ferrous salts. Other reducing agents are described in
Brodack et. al., PCT Application 94/22496, which is
incorporated herein by reference.

A "transfer ligand" is a ligand that forms an
intermediate complex with a metal ion that is stable enough
30 to prevent unwanted side-reactions but labile enough to be
converted to a metallopharmaceutical. The formation of the
intermediate complex is kinetically favored while the
formation of the metallopharmaceutical is thermodynamically
favored. Transfer ligands useful in the preparation of
35 metallopharmaceuticals and in diagnostic kits useful for
the preparation of diagnostic radiopharmaceuticals include
but are not limited to gluconate, glucoheptonate, mannitol,
glucarate, N,N,N',N'-ethylenediaminetetraacetic acid,

pyrophosphate and methylenediphosphonate. In general, transfer ligands are comprised of oxygen or nitrogen donor atoms.

5 The term "donor atom" refers to the atom directly attached to a metal by a chemical bond.

"Ancillary" or "co-ligands" are ligands that are incorporated into a radiopharmaceutical during its synthesis. They serve to complete the coordination sphere of the radionuclide together with the chelator or
10 radionuclide bonding unit of the reagent. For radiopharmaceuticals comprised of a binary ligand system, the radionuclide coordination sphere is composed of one or more chelators or bonding units from one or more reagents and one or more ancillary or co-ligands, provided that
15 there are a total of two types of ligands, chelators or bonding units. For example, a radiopharmaceutical comprised of one chelator or bonding unit from one reagent and two of the same ancillary or co-ligands and a radiopharmaceutical comprised of two chelators or bonding
20 units from one or two reagents and one ancillary or co-ligand are both considered to be comprised of binary ligand systems. For radiopharmaceuticals comprised of a ternary ligand system, the radionuclide coordination sphere is composed of one or more chelators or bonding units from
25 one or more reagents and one or more of two different types of ancillary or co-ligands, provided that there are a total of three types of ligands, chelators or bonding units. For example, a radiopharmaceutical comprised of one chelator or bonding unit from one reagent and two different ancillary
30 or co-ligands is considered to be comprised of a ternary ligand system.

Ancillary or co-ligands useful in the preparation of radiopharmaceuticals and in diagnostic kits useful for the
preparation of said radiopharmaceuticals are comprised of
35 one or more oxygen, nitrogen, carbon, sulfur, phosphorus, arsenic, selenium, and tellurium donor atoms. A ligand can be a transfer ligand in the synthesis of a radiopharmaceutical and also serve as an ancillary or

co-ligand in another radiopharmaceutical. Whether a ligand is termed a transfer or ancillary or co-ligand depends on whether the ligand remains in the radionuclide coordination sphere in the radiopharmaceutical, which is determined by
5 the coordination chemistry of the radionuclide and the chelator or bonding unit of the reagent or reagents.

A "chelator" or "bonding unit" is the moiety or group on a reagent that binds to a metal ion through the formation of chemical bonds with one or more donor atoms.

10 A "diagnostic kit" or "kit" comprises a collection of components, termed the formulation, in one or more vials which are used by the practicing end user in a clinical or pharmacy setting to synthesize diagnostic
15 radiopharmaceuticals. The kit provides all the requisite components to synthesize and use the diagnostic radiopharmaceutical except those that are commonly available to the practicing end user, such as water or saline for injection, a solution of the radionuclide, equipment for heating the kit during the synthesis of the
20 radiopharmaceutical, if required, equipment necessary for administering the radiopharmaceutical to the patient such as syringes and shielding, and imaging equipment.

A "lyophilization aid" is a component that has favorable physical properties for lyophilization, such as
25 the glass transition temperature, and is added to the formulation to improve the physical properties of the combination of all the components of the formulation for lyophilization.

A "stabilization aid" is a component that is added to
30 the metallopharmaceutical or to the diagnostic kit either to stabilize the metallopharmaceutical or to prolong the shelf-life of the kit before it must be used. Stabilization aids can be antioxidants, reducing agents or radical scavengers and can provide improved stability by
35 reacting preferentially with species that degrade other components or the metallopharmaceutical.

A "solubilization aid" is a component that improves the solubility of one or more other components in the medium required for the formulation.

5 A "bacteriostat" is a component that inhibits the growth of bacteria in a formulation either during its storage before use or after a diagnostic kit is used to synthesize a radiopharmaceutical.

10 As used herein, the term "bubbles", as used herein, refers to vesicles which are generally characterized by the presence of one or more membranes or walls surrounding an internal void that is filled with a gas or precursor thereto. Exemplary bubbles include, for example, liposomes, micelles and the like.

15 As used herein, the term "lipid" refers to a synthetic or naturally-occurring amphipathic compound which comprises a hydrophilic component and a hydrophobic component. Lipids include, for example, fatty acids, neutral fats, phosphatides, glycolipids, aliphatic alcohols and waxes, terpenes and steroids.

20 As used herein, the term "lipid composition" refers to a composition which comprises a lipid compound. Exemplary lipid compositions include suspensions, emulsions and vesicular compositions.

25 As used herein, the term "lipid formulation" refers to a composition which comprises a lipid compound and a bioactive agent.

30 As used herein, the term "vesicle" refers to a spherical entity which is characterized by the presence of an internal void. Preferred vesicles are formulated from lipids, including the various lipids described herein. In any given vesicle, the lipids may be in the form of a monolayer or bilayer, and the mono- or bilayer lipids may be used to form one or more mono- or bilayers. In the case of more than one mono- or bilayer, the mono- or bilayers are generally concentric. The lipid vesicles described
35 herein include such entities commonly referred to as liposomes, micelles, bubbles, microbubbles, microspheres and the like. Thus, the lipids may be used to form a

unilamellar vesicle (comprised of one monolayer or bilayer), an oligolamellar vesicle (comprised of about two or about three monolayers or bilayers) or a multilamellar vesicle (comprised of more than about three monolayers or bilayers). The internal void of the vesicles may be filled with a liquid, including, for example, an aqueous liquid, a gas, a gaseous precursor, and/or a solid or solute material, including, for example, a bioactive agent, as desired.

10 As used herein, the term "vesicular composition" refers to a composition which is formulate from lipids and which comprises vesicles.

As used herein, the term "vesicle formulation" refers to a composition which comprises vesicles and a bioactive agent.

15 As used herein, the term "liposomes" refers to a generally spherical cluster or aggregate of amphipathic compounds, including lipid compounds, typically in the form of one or more concentric layers, for example, bilayers.

20 They may also be referred to herein as lipid vesicles.

The ultrasound contrast agents of the present invention comprise a plurality of inhibitors of beta-amyloid production attached to or incorporated into a microbubble of a biocompatible gas, a liquid carrier, and a surfactant microsphere, further comprising an optional linking moiety, L_n , between the targeting moieties and the microbubble. In this context, the term liquid carrier means aqueous solution and the term surfactant means any amphiphilic material which produces a reduction in interfacial tension in a solution. A list of suitable surfactants for forming surfactant microspheres is disclosed in EP0727225A2, herein incorporated by reference. The term surfactant microsphere includes nanospheres, liposomes, vesicles and the like. The biocompatible gas can be air, or a fluorocarbon, such as a C₃-C₅ perfluoroalkane, which provides the difference in echogenicity and thus the contrast in ultrasound imaging.

The gas is encapsulated or contained in the microsphere to which is attached the biodirecting group, optionally via a linking group. The attachment can be covalent, ionic or by van der Waals forces. Specific examples of such contrast agents include lipid encapsulated perfluorocarbons with a plurality inhibitors of beta-amyloid production.

X-ray contrast agents of the present invention are comprised of one or more inhibitors of beta-amyloid production attached to one or more X-ray absorbing or "heavy" atoms of atomic number 20 or greater, further comprising an optional linking moiety, L_n , between the targeting moieties and the X-ray absorbing atoms. The frequently used heavy atom in X-ray contrast agents is iodine. Recently, X-ray contrast agents comprised of metal chelates (Wallace, R., U.S. 5,417,959) and polychelates comprised of a plurality of metal ions (Love, D., U.S. 5,679,810) have been disclosed. More recently, multinuclear cluster complexes have been disclosed as X-ray contrast agents (U.S. 5,804,161, PCT WO91/14460, and PCT WO 92/17215).

MRI contrast agents of the present invention are comprised of one or more inhibitors of beta-amyloid production attached to one or more paramagnetic metal ions, further comprising an optional linking moiety, L_n , between the targeting moieties and the paramagnetic metal ions. The paramagnetic metal ions are present in the form of metal complexes or metal oxide particles. U.S. 5,412,148, and 5,760,191, describe examples of chelators for paramagnetic metal ions for use in MRI contrast agents. U.S. 5,801,228, U.S. 5,567,411, and U.S. 5,281,704, describe examples of polychelants useful for complexing more than one paramagnetic metal ion for use in MRI contrast agents. U.S. 5,520,904, describes particulate compositions comprised of paramagnetic metal ions for use as MRI contrast agents.

The pharmaceuticals of the present invention have the formulae, $(Q^3)_d-L_n-(Ch-X)$, $(Q^3)_d-L_n-(Ch-X^1)_d'$, $(Q^3)_d-L_n-(X^2)_d''$, and $(Q^3)_d-L_n-(X^3)$, wherein Q^3 represents an

inhibitor of beta-amyloid production, d is 1-10, L_n represents an optional linking group, Ch represents a metal chelator or bonding moiety, X represents a radioisotope, X^1 represents paramagnetic metal ion, X^2 represents a
5 paramagnetic metal ion or heavy atom containing insoluble solid particle, d'' is 1-100, and X^3 represents a surfactant microsphere of an echogenic gas.

The pharmaceuticals of the present invention can be synthesized by several approaches. One approach involves
10 the synthesis of the inhibitor of beta-amyloid production, Q^3 , and direct attachment of one or more moieties, Q^3 , to one or more metal chelators or bonding moieties, Ch , or to a paramagnetic metal ion or heavy atom containing solid particle, or to an echogenic gas microbubble. Another
15 approach involves the attachment of one or more moieties, Q^3 , to the linking group, L_n , which is then attached to one or more metal chelators or bonding moieties, Ch , or to a paramagnetic metal ion or heavy atom containing solid particle, or to an echogenic gas microbubble. Another
20 approach involves the synthesis of an inhibitor of beta-amyloid production, Q^3 , bearing a fragment of the linking group, L_n , one or more of which are then attached to the remainder of the linking group and then to one or more metal chelators or bonding moieties, Ch , or to a
25 paramagnetic metal ion or heavy atom containing solid particle, or to an echogenic gas microbubble.

The inhibitor of beta-amyloid production, Q^3 , optionally bearing a linking group, L_n , or a fragment of the linking group, can be synthesized using standard
30 synthetic methods known to those skilled in the art. Preferred methods include but are not limited to those methods described below.

The attachment of linking groups, L_n , to the inhibitor of beta-amyloid production, Q^3 ; chelators or bonding units, Ch , to the inhibitor of beta-amyloid production, Q^3 , or to
35 the linking groups, L_n ; and non-peptides, bearing a fragment of the linking group to the remainder of the linking group, in combination forming the moiety, $(Q^3)_d-L_n$,

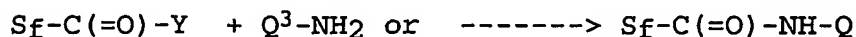
and then to the moiety CH_2 ; can all be performed by standard techniques. These include, but are not limited to, amidation, esterification, alkylation, and the formation of ureas or thioureas. Procedures for performing these
 5 attachments can be found in Brinkley, M., *Bioconjugate Chemistry* 1992, 3(1), which is incorporated herein by reference.

A number of methods can be used to attach the inhibitor of beta-amyloid production, Q^3 , to paramagnetic
 10 metal ion or heavy atom containing solid particles, X^2 , by one of skill in the art of the surface modification of solid particles. In general, the inhibitor of beta-amyloid production Q^3 or the combination $(Q^3)_dL_n$ is attached to a
 15 coupling group that react with a constituent of the surface of the solid particle. The coupling groups can be any of a number of silanes which react with surface hydroxyl groups on the solid particle surface, as described in co-pending United States Patent Application Serial No. 09/356,178, and can also include polyphosphonates, polycarboxylates,
 20 polyphosphates or mixtures thereof which couple with the surface of the solid particles, as described in U.S. 5,520,904.

A number of reaction schemes can be used to attach the inhibitor of beta-amyloid production, Q^3 , to the surfactant
 25 microsphere, X^3 . These are illustrated in following reaction schemes where S_f represents a surfactant moiety that forms the surfactant microsphere.

Acylation Reaction:

30



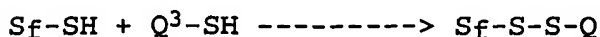
or



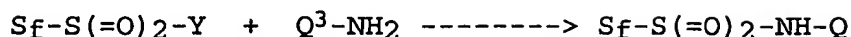
wherein Y is a leaving group or active ester

35

Disulfide Coupling:

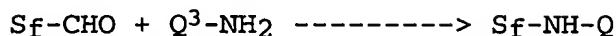


Sulfonamide Coupling:



5

Reductive Amidation:



10 In these reaction schemes, the substituents Sf and Q can be reversed as well.

The linking group L_n can serve several roles. First it provides a spacing group between the metal chelator or bonding moiety, C_h , the paramagnetic metal ion or heavy atom containing solid particle, X^2 , and the surfactant microsphere, X^3 , and the one or more of the inhibitors of beta-amyloid production, Q^3 , so as to minimize the possibility that the moieties $C_h\text{-X}$, $C_h\text{-X}^1$, X^2 , and X^3 , will interfere with the interaction of the recognition site of Q^3 with receptor associated with beta-amyloid production. The necessity of incorporating a linking group in a reagent is dependent on the identity of Q^3 , $C_h\text{-X}$, $C_h\text{-X}^1$, X^2 , and X^3 . If $C_h\text{-X}$, $C_h\text{-X}^1$, X^2 , and X^3 , cannot be attached to Q without substantially diminishing its affinity for the receptors, then a linking group is used. A linking group also provides a means of independently attaching multiple non-peptides, Q^3 , to one group that is attached to $C_h\text{-X}$, $C_h\text{-X}^1$, X^2 , or X^3 .

The linking group also provides a means of incorporating a pharmacokinetic modifier into the pharmaceuticals of the present invention. The pharmacokinetic modifier serves to direct the biodistribution of the injected pharmaceutical other than by the interaction of the inhibitor of beta-amyloid production, Q^3 , with the receptor associated with beta-amyloid production. A wide variety of functional groups can serve as pharmacokinetic modifiers, including, but not limited to, carbohydrates, polyalkylene glycols, peptides

or other polyamino acids, and cyclodextrins. The modifiers can be used to enhance or decrease hydrophilicity and to enhance or decrease the rate of blood clearance. The modifiers can also be used to direct the route of
5 elimination of the pharmaceuticals. Preferred pharmacokinetic modifiers are those that result in moderate to fast blood clearance and enhanced renal excretion.

The metal chelator or bonding moiety, Ch , is selected to form stable complexes with the metal ion chosen for the
10 particular application. Chelators or bonding moieties for diagnostic radiopharmaceuticals are selected to form stable complexes with the radioisotopes that have imageable gamma ray or positron emissions, such as ^{99m}Tc , ^{95}Tc , ^{111}In , ^{62}Cu , ^{60}Cu , ^{64}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y .

15 Chelators for technetium, copper and gallium isotopes are selected from diaminedithiols, monoamine-monoamidedithiols, triamide-monothiol, monoamine-diamide-monothiol, diaminedioximes, and hydrazines. The chelators are generally tetradentate with
20 donor atoms selected from nitrogen, oxygen and sulfur. Preferred reagents are comprised of chelators having amine nitrogen and thiol sulfur donor atoms and hydrazine bonding units. The thiol sulfur atoms and the hydrazines may bear a protecting group which can be displaced either prior to
25 using the reagent to synthesize a radiopharmaceutical or preferably in situ during the synthesis of the radiopharmaceutical.

Exemplary thiol protecting groups include those listed in Greene and Wuts, "Protective Groups in Organic
30 Synthesis" John Wiley & Sons, New York (1991), the disclosure of which is hereby incorporated by reference. Any thiol protecting group known in the art can be used. Examples of thiol protecting groups include, but are not limited to, the following: acetamidomethyl,
35 benzamidomethyl, 1-ethoxyethyl, benzoyl, and triphenylmethyl.

Exemplary protecting groups for hydrazine bonding units are hydrazones which can be aldehyde or ketone

hydrazones having substituents selected from hydrogen, alkyl, aryl and heterocycle. Particularly preferred hydrazones are described in co-pending U.S.S.N. 08/476,296 the disclosure of which is herein incorporated by reference
5 in its entirety.

The hydrazine bonding unit when bound to a metal radionuclide is termed a hydrazido, or diazenido group and serves as the point of attachment of the radionuclide to the remainder of the radiopharmaceutical. A diazenido group
10 can be either terminal (only one atom of the group is bound to the radionuclide) or chelating. In order to have a chelating diazenido group at least one other atom of the group must also be bound to the radionuclide. The atoms bound to the metal are termed donor atoms.

15 Chelators for ^{111}In and ^{86}Y are selected from cyclic and acyclic polyaminocarboxylates such as DTPA, DOTA, DO3A, 2-benzyl-DOTA, alpha-(2-phenethyl)1,4,7,10-tetraazacyclododecane-1-acetic-4,7,10-tris(methylacetic)acid, 2-benzyl-
20 cyclohexyldiethylenetriaminepentaacetic acid, 2-benzyl-6-methyl-DTPA, and 6,6"-bis[N,N,N",N"-tetra(carboxymethyl)aminomethyl)-4'-(3-amino-4-methoxyphenyl)-2,2':6',2"-terpyridine. Procedures for synthesizing these chelators that are not commercially
25 available can be found in Brechbiel, M. and Gansow, O., *J. Chem. Soc. Perkin Trans.* **1992**, 1, 1175; Brechbiel, M. and Gansow, O., *Bioconjugate Chem.* **1991**, 2, 187; Deshpande, S., et. al., *J. Nucl. Med.* **1990**, 31, 473; Kruper, J., U.S. Patent 5,064,956, and Toner, J., U.S. Patent 4,859,777, the
30 disclosures of which are hereby incorporated by reference in their entirety.

The coordination sphere of metal ion includes all the ligands or groups bound to the metal. For a transition metal radionuclide to be stable it typically has a
35 coordination number (number of donor atoms) comprised of an integer greater than or equal to 4 and less than or equal to 8; that is there are 4 to 8 atoms bound to the metal and it is said to have a complete coordination sphere. The

requisite coordination number for a stable radionuclide complex is determined by the identity of the radionuclide, its oxidation state, and the type of donor atoms. If the chelator or bonding unit does not provide all of the atoms
5 necessary to stabilize the metal radionuclide by completing its coordination sphere, the coordination sphere is completed by donor atoms from other ligands, termed ancillary or co-ligands, which can also be either terminal or chelating.

10 A large number of ligands can serve as ancillary or co-ligands, the choice of which is determined by a variety of considerations such as the ease of synthesis of the radiopharmaceutical, the chemical and physical properties of the ancillary ligand, the rate of formation, the yield,
15 and the number of isomeric forms of the resulting radiopharmaceuticals, the ability to administer said ancillary or co-ligand to a patient without adverse physiological consequences to said patient, and the compatibility of the ligand in a lyophilized kit
20 formulation. The charge and lipophilicity of the ancillary ligand will effect the charge and lipophilicity of the radiopharmaceuticals. For example, the use of 4,5-dihydroxy-1,3-benzene disulfonate results in radiopharmaceuticals with an additional two anionic groups
25 because the sulfonate groups will be anionic under physiological conditions. The use of N-alkyl substituted 3,4-hydroxypyridinones results in radiopharmaceuticals with varying degrees of lipophilicity depending on the size of the alkyl substituents.

30 Preferred technetium radiopharmaceuticals of the present invention are comprised of a hydrazido or diazenido bonding unit and an ancillary ligand, A_{L1} , or a bonding unit and two types of ancillary A_{L1} and A_{L2} , or a
35 tetradentate chelator comprised of two nitrogen and two sulfur atoms. Ancillary ligands A_{L1} are comprised of two or more hard donor atoms such as oxygen and amine nitrogen (sp^3 hybridized). The donor atoms occupy at least two of the sites in the coordination sphere of the radionuclide

metal; the ancillary ligand A_{L1} serves as one of the three ligands in the ternary ligand system. Examples of ancillary ligands A_{L1} include but are not limited to dioxygen ligands and functionalized aminocarboxylates. A large number of such ligands are available from commercial sources.

Ancillary dioxygen ligands include ligands that coordinate to the metal ion through at least two oxygen donor atoms. Examples include but are not limited to: glucoheptonate, gluconate, 2-hydroxyisobutyrate, lactate, tartrate, mannitol, glucarate, maltol, Kojic acid, 2,2-bis(hydroxymethyl)propionic acid, 4,5-dihydroxy-1,3-benzene disulfonate, or substituted or unsubstituted 1,2 or 3,4 hydroxypyridinones. (The names for the ligands in these examples refer to either the protonated or non-protonated forms of the ligands.)

Functionalized aminocarboxylates include ligands that have a combination of amine nitrogen and oxygen donor atoms. Examples include but are not limited to: iminodiacetic acid, 2,3-diaminopropionic acid, nitrilotriacetic acid, N,N'-ethylenediamine diacetic acid, N,N,N'-ethylenediamine triacetic acid, hydroxyethylethylenediamine triacetic acid, and N,N'-ethylenediamine bis-hydroxyphenylglycine. (The names for the ligands in these examples refer to either the protonated or non-protonated forms of the ligands.)

A series of functionalized aminocarboxylates are disclosed by Bridger et. al. in U.S. Patent 5,350,837, herein incorporated by reference, that result in improved rates of formation of technetium labeled hydrazino modified proteins. We have determined that certain of these aminocarboxylates result in improved yields of the radiopharmaceuticals of the present invention. The preferred ancillary ligands A_{L1} functionalized aminocarboxylates that are derivatives of glycine; the most preferred is tricine (tris(hydroxymethyl)methylglycine).

The most preferred technetium radiopharmaceuticals of the present invention are comprised of a hydrazido or

diazenido bonding unit and two types of ancillary designated A_{L1} and A_{L2} , or a diaminedithiol chelator. The second type of ancillary ligands A_{L2} are comprised of one or more soft donor atoms selected from the group: phosphine
5 phosphorus, arsine arsenic, imine nitrogen (sp^2 hybridized), sulfur (sp^2 hybridized) and carbon (sp hybridized); atoms which have p-acid character. Ligands A_{L2} can be monodentate, bidentate or tridentate, the denticity is defined by the number of donor atoms in the
10 ligand. One of the two donor atoms in a bidentate ligand and one of the three donor atoms in a tridentate ligand must be a soft donor atom. We have disclosed in co-pending U.S.S.N. 08/415,908, and U.S.S.N. 60/013360 and 08/646,886, the disclosures of which are herein incorporated by
15 reference in their entirety, that radiopharmaceuticals comprised of one or more ancillary or co-ligands A_{L2} are more stable compared to radiopharmaceuticals that are not comprised of one or more ancillary ligands, A_{L2} ; that is, they have a minimal number of isomeric forms, the relative
20 ratios of which do not change significantly with time, and that remain substantially intact upon dilution.

The ligands A_{L2} that are comprised of phosphine or arsine donor atoms are trisubstituted phosphines, trisubstituted arsines, tetrasubstituted diphosphines and
25 tetrasubstituted diarsines. The ligands A_{L2} that are comprised of imine nitrogen are unsaturated or aromatic nitrogen-containing, 5 or 6-membered heterocycles. The ligands that are comprised of sulfur (sp^2 hybridized) donor atoms are thiocarbonyls, comprised of the moiety $C=S$. The
30 ligands comprised of carbon (sp hybridized) donor atoms are isonitriles, comprised of the moiety CNR , where R is an organic radical. A large number of such ligands are available from commercial sources. Isonitriles can be synthesized as described in European Patent 0107734 and in
35 U.S. Patent 4,988,827, herein incorporated by reference.

Preferred ancillary ligands A_{L2} are trisubstituted phosphines and unsaturated or aromatic 5 or 6 membered heterocycles. The most preferred ancillary ligands A_{L2} are

trisubstituted phosphines and unsaturated 5 membered heterocycles.

The ancillary ligands AL_2 may be substituted with alkyl, aryl, alkoxy, heterocycle, aralkyl, alkaryl and arylalkaryl groups and may or may not bear functional groups comprised of heteroatoms such as oxygen, nitrogen, phosphorus or sulfur. Examples of such functional groups include but are not limited to: hydroxyl, carboxyl, carboxamide, nitro, ether, ketone, amino, ammonium, sulfonate, sulfonamide, phosphonate, and phosphonamide. The functional groups may be chosen to alter the lipophilicity and water solubility of the ligands which may affect the biological properties of the radiopharmaceuticals, such as altering the distribution into non-target tissues, cells or fluids, and the mechanism and rate of elimination from the body.

Chelators for magnetic resonance imaging contrast agents are selected to form stable complexes with paramagnetic metal ions, such as Gd(III), Dy(III), Fe(III), and Mn(II), are selected from cyclic and acyclic polyaminocarboxylates such as DTPA, DOTA, DO3A, 2-benzyl-DOTA, alpha-(2-phenethyl)1,4,7,10-tetraazacyclododecane-1-acetic-4,7,10-tris(methylacetic)acid, 2-benzyl-cyclohexyldiethylenetriaminepentaacetic acid, 2-benzyl-6-methyl-DTPA, and 6,6"-bis[N,N,N",N"-tetra(carboxymethyl)aminomethyl)-4'-(3-amino-4-methoxyphenyl)-2,2':6',2"-terpyridine.

The technetium radiopharmaceuticals of the present invention comprised of a hydrazido or diazenido bonding unit can be easily prepared by admixing a salt of a radionuclide, a reagent of the present invention, an ancillary ligand AL_1 , an ancillary ligand AL_2 , and a reducing agent, in an aqueous solution at temperatures from 0 to 100 °C. The technetium radiopharmaceuticals of the present invention comprised of a tetradentate chelator having two nitrogen and two sulfur atoms can be easily prepared by admixing a salt of a radionuclide, a reagent of

the present invention, and a reducing agent, in an aqueous solution at temperatures from 0 to 100 °C.

When the bonding unit in the reagent of the present invention is present as a hydrazone group, then it must first be converted to a hydrazine, which may or may not be protonated, prior to complexation with the metal radionuclide. The conversion of the hydrazone group to the hydrazine can occur either prior to reaction with the radionuclide, in which case the radionuclide and the ancillary or co-ligand or ligands are combined not with the reagent but with a hydrolyzed form of the reagent bearing the chelator or bonding unit, or in the presence of the radionuclide in which case the reagent itself is combined with the radionuclide and the ancillary or co-ligand or ligands. In the latter case, the pH of the reaction mixture must be neutral or acidic.

Alternatively, the radiopharmaceuticals of the present invention comprised of a hydrazido or diazenido bonding unit can be prepared by first admixing a salt of a radionuclide, an ancillary ligand A_{L1} , and a reducing agent in an aqueous solution at temperatures from 0 to 100 °C to form an intermediate radionuclide complex with the ancillary ligand A_{L1} then adding a reagent of the present invention and an ancillary ligand A_{L2} and reacting further at temperatures from 0 to 100 °C.

Alternatively, the radiopharmaceuticals of the present invention comprised of a hydrazido or diazenido bonding unit can be prepared by first admixing a salt of a radionuclide, an ancillary ligand A_{L1} , a reagent of the present invention, and a reducing agent in an aqueous solution at temperatures from 0 to 100 °C to form an intermediate radionuclide complex, and then adding an ancillary ligand A_{L2} and reacting further at temperatures from 0 to 100 °C.

The technetium radionuclide is preferably in the chemical form of pertechnetate or perrhenate and a pharmaceutically acceptable cation. The pertechnetate salt form is preferably sodium pertechnetate such as obtained

from commercial Tc-99m generators. The amount of pertechnetate used to prepare the radiopharmaceuticals of the present invention can range from 0.1 mCi to 1 Ci, or more preferably from 1 to 200 mCi.

5 The amount of the reagent of the present invention used to prepare the technetium radiopharmaceutical of the present invention can range from 0.01 μ g to 10 mg, or more preferably from 0.5 μ g to 200 μ g. The amount used will be dictated by the amounts of the other reactants and the
10 identity of the radiopharmaceuticals of the present invention to be prepared.

 The amounts of the ancillary ligands A_{L1} used can range from 0.1 mg to 1 g, or more preferably from 1 mg to 100 mg. The exact amount for a particular
15 radiopharmaceutical is a function of identity of the radiopharmaceuticals of the present invention to be prepared, the procedure used and the amounts and identities of the other reactants. Too large an amount of A_{L1} will result in the formation of by-products comprised of
20 technetium labeled A_{L1} without a biologically active molecule or by-products comprised of technetium labeled biologically active molecules with the ancillary ligand A_{L1} but without the ancillary ligand A_{L2} . Too small an amount of A_{L1} will result in other by-products such as technetium
25 labeled biologically active molecules with the ancillary ligand A_{L2} but without the ancillary ligand A_{L1} , or reduced hydrolyzed technetium, or technetium colloid.

 The amounts of the ancillary ligands A_{L2} used can range from 0.001 mg to 1 g, or more preferably from 0.01 mg
30 to 10 mg. The exact amount for a particular radiopharmaceutical is a function of the identity of the radiopharmaceuticals of the present invention to be prepared, the procedure used and the amounts and identities of the other reactants. Too large an amount of A_{L2} will
35 result in the formation of by-products comprised of technetium labeled A_{L2} without a biologically active molecule or by-products comprised of technetium labeled biologically active molecules with the ancillary ligand A_{L2}

but without the ancillary ligand A_{L1} . If the reagent bears one or more substituents that are comprised of a soft donor atom, as defined above, at least a ten-fold molar excess of the ancillary ligand A_{L2} to the reagent of formula 2 is
5 required to prevent the substituent from interfering with the coordination of the ancillary ligand A_{L2} to the metal radionuclide.

Suitable reducing agents for the synthesis of the radiopharmaceuticals of the present invention include
10 stannous salts, dithionite or bisulfite salts, borohydride salts, and formamidinesulfinic acid, wherein the salts are of any pharmaceutically acceptable form. The preferred reducing agent is a stannous salt. The amount of a reducing agent used can range from 0.001 mg to 10 mg, or
15 more preferably from 0.005 mg to 1 mg.

The specific structure of a radiopharmaceutical of the present invention comprised of a hydrazido or diazenido bonding unit will depend on the identity of the reagent of the present invention used, the identity of any ancillary
20 ligand A_{L1} , the identity of any ancillary ligand A_{L2} , and the identity of the radionuclide. Radiopharmaceuticals comprised of a hydrazido or diazenido bonding unit synthesized using concentrations of reagents of $<100 \mu\text{g/mL}$, will be comprised of one hydrazido or diazenido group.
25 Those synthesized using $>1 \text{ mg/mL}$ concentrations will be comprised of two hydrazido or diazenido groups from two reagent molecules. For most applications, only a limited amount of the biologically active molecule can be injected and not result in undesired side-effects, such as chemical
30 toxicity, interference with a biological process or an altered biodistribution of the radiopharmaceutical. Therefore, the radiopharmaceuticals which require higher concentrations of the reagents comprised in part of the biologically active molecule, will have to be diluted or
35 purified after synthesis to avoid such side-effects.

The identities and amounts used of the ancillary ligands A_{L1} and A_{L2} will determine the values of the variables y and z . The values of y and z can independently

be an integer from 1 to 2. In combination, the values of y and z will result in a technetium coordination sphere that is made up of at least five and no more than seven donor atoms. For monodentate ancillary ligands AL_2 , z can be an integer from 1 to 2; for bidentate or tridentate ancillary ligands AL_2 , z is 1. The preferred combination for monodentate ligands is y equal to 1 or 2 and z equal to 1. The preferred combination for bidentate or tridentate ligands is y equal to 1 and z equal to 1.

10 The indium, copper, and gallium radiopharmaceuticals of the present invention can be easily prepared by admixing a salt of a radionuclide and a reagent of the present invention, in an aqueous solution at temperatures from 0 to 100 °C. These radionuclides are typically obtained as a dilute aqueous solution in a mineral acid, such as hydrochloric, nitric or sulfuric acid. The radionuclides are combined with from one to about one thousand equivalents of the reagents of the present invention dissolved in aqueous solution. A buffer is typically used to maintain the pH of the reaction mixture between 3 and 10.

25 The gadolinium, dysprosium, iron and manganese metallopharmaceuticals of the present invention can be easily prepared by admixing a salt of the paramagnetic metal ion and a reagent of the present invention, in an aqueous solution at temperatures from 0 to 100 °C. These paramagnetic metal ions are typically obtained as a dilute aqueous solution in a mineral acid, such as hydrochloric, nitric or sulfuric acid. The paramagnetic metal ions are combined with from one to about one thousand equivalents of the reagents of the present invention dissolved in aqueous solution. A buffer is typically used to maintain the pH of the reaction mixture between 3 and 10.

35 The total time of preparation will vary depending on the identity of the metal ion, the identities and amounts of the reactants and the procedure used for the preparation. The preparations may be complete, resulting in > 80% yield of the radiopharmaceutical, in 1 minute or

may require more time. If higher purity metallopharmaceuticals are needed or desired, the products can be purified by any of a number of techniques well known to those skilled in the art such as liquid chromatography, solid phase extraction, solvent extraction, dialysis or ultrafiltration.

Buffers useful in the preparation of metallopharmaceuticals and in diagnostic kits useful for the preparation of said radiopharmaceuticals include but are not limited to phosphate, citrate, sulfosalicylate, and acetate. A more complete list can be found in the United States Pharmacopeia.

Lyophilization aids useful in the preparation of diagnostic kits useful for the preparation of radiopharmaceuticals include but are not limited to mannitol, lactose, sorbitol, dextran, Ficoll, and polyvinylpyrrolidone (PVP).

Stabilization aids useful in the preparation of metallopharmaceuticals and in diagnostic kits useful for the preparation of radiopharmaceuticals include but are not limited to ascorbic acid, cysteine, monothioglycerol, sodium bisulfite, sodium metabisulfite, gentisic acid, and inositol.

Solubilization aids useful in the preparation of metallopharmaceuticals and in diagnostic kits useful for the preparation of radiopharmaceuticals include but are not limited to ethanol, glycerin, polyethylene glycol, propylene glycol, polyoxyethylene sorbitan monooleate, sorbitan monooleate, polysorbates, poly(oxyethylene)poly(oxypropylene)poly(oxyethylene) block copolymers (Pluronic) and lecithin. Preferred solubilizing aids are polyethylene glycol, and Pluronic.

Bacteriostats useful in the preparation of metallopharmaceuticals and in diagnostic kits useful for the preparation of radiopharmaceuticals include but are not limited to benzyl alcohol, benzalkonium chloride, chlorbutanol, and methyl, propyl or butyl paraben.

A component in a diagnostic kit can also serve more than one function. A reducing agent can also serve as a stabilization aid, a buffer can also serve as a transfer ligand, a lyophilization aid can also serve as a transfer, ancillary or co-ligand and so forth.

The diagnostic radiopharmaceuticals are administered by intravenous injection, usually in saline solution, at a dose of 1 to 100 mCi per 70 kg body weight, or preferably at a dose of 5 to 50 mCi. Imaging is performed using known procedures.

The magnetic resonance imaging contrast agents of the present invention may be used in a similar manner as other MRI agents as described in U.S. Patent 5,155,215; U.S. Patent 5,087,440; Margerstadt et al., Magn. Reson. Med., 1986, 3, 808; Runge et al., Radiology, 1988, 166, 835; and Bousquet et al., Radiology, 1988, 166, 693. Generally, sterile aqueous solutions of the contrast agents are administered to a patient intravenously in dosages ranging from 0.01 to 1.0 mmoles per kg body weight.

For use as X-ray contrast agents, the compositions of the present invention should generally have a heavy atom concentration of 1 mM to 5 M, preferably 0.1 M to 2 M. Dosages, administered by intravenous injection, will typically range from 0.5 mmol/kg to 1.5 mmol/kg, preferably 0.8 mmol/kg to 1.2 mmol/kg. Imaging is performed using known techniques, preferably X-ray computed tomography.

The ultrasound contrast agents of the present invention are administered by intravenous injection in an amount of 10 to 30 μ L of the echogenic gas per kg body weight or by infusion at a rate of approximately 3 μ L/kg/min. Imaging is performed using known techniques of sonography.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

SYNTHESIS

The compounds of the present invention can be prepared in a number of ways well known to one skilled in the art of organic synthesis. The compounds of the present invention can be synthesized using the methods described below,
5 together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. Preferred methods include, but are not limited to, those described below. All references cited herein are hereby incorporated in
10 their entirety herein by reference.

The novel compounds of this invention may be prepared using the reactions and techniques described in this section. The reactions are performed in solvents appropriate to the reagents and materials employed and are
15 suitable for the transformations being effected. Also, in the description of the synthetic methods described below, it is to be understood that all proposed reaction conditions, including choice of solvent, reaction atmosphere, reaction temperature, duration of the
20 experiment and workup procedures, are chosen to be the conditions standard for that reaction, which should be readily recognized by one skilled in the art. It is understood by one skilled in the art of organic synthesis that the functionality present on various portions of the
25 molecule must be compatible with the reagents and reactions proposed. Such restrictions to the substituents which are compatible with the reaction conditions will be readily apparent to one skilled in the art and alternate methods must then be used.

30 Methods for the synthesis of succinylamino lactams are known in the art and are disclosed in a number of references including PCT publication number WO 96/29313, which is hereby incorporated by reference.

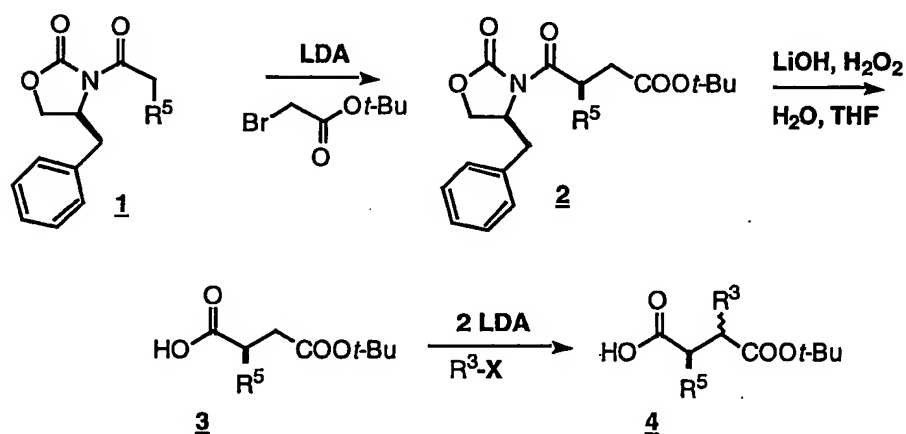
Disubstituted succinate derivatives can be prepared by
35 a number of known procedures. The procedure of Evans (D. A. Evans et al, *Org. Synth.* **86**, p83 (1990)) is outlined in Scheme 1 where acylation of an oxazolidinone with an acylating agent such as an acid chloride provides

structures 1. Alkylation to form 2 followed by cleavage of the chiral auxiliary and subsequent alkylation of the dianion of the carboxylic acid 3 provides a variety of

5 incorporated into structures of Formula (I) by those skilled in the art. Additional examples are found in P. Becket, M. J. Crimmin, M. H. Davis, Z. Spavold, Synlett, (1993), 137-138, incorporated herein by reference.

10

Scheme 1



Diastereomerically pure succinate derivatives can be

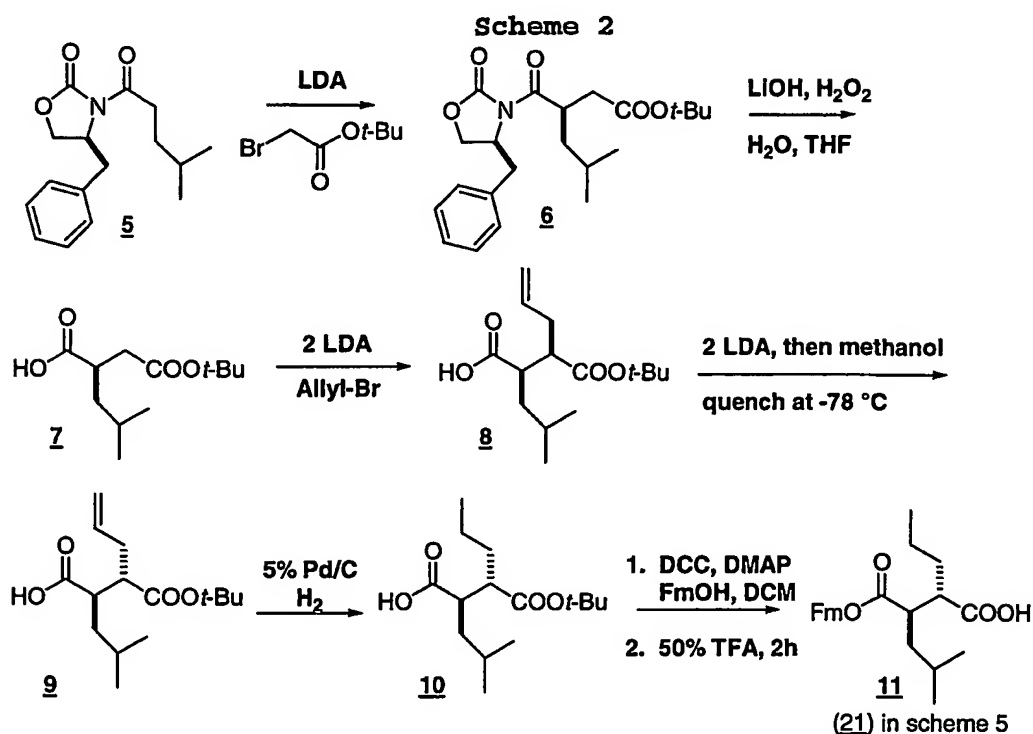
15 accessed using the chemistry outlined below, adapted from P. Becket, M. J. Crimmin, M. H. Davis, Z. Spavold, Synlett, (1993), 137-138 incorporated herein by reference. This reference provides the synthesis below to obtain compound

9. Compound 11 is used as an intermediate and is prepared

20 from 9 by hydrogenation of the allyl group followed by coupling of 9-fluorenmethanol under standard conditions using DCC and DMAP in CH₂Cl₂. Deprotection of the *tert*-butyl ester is accomplished by treatment with 50% trifluoroacetic acid.

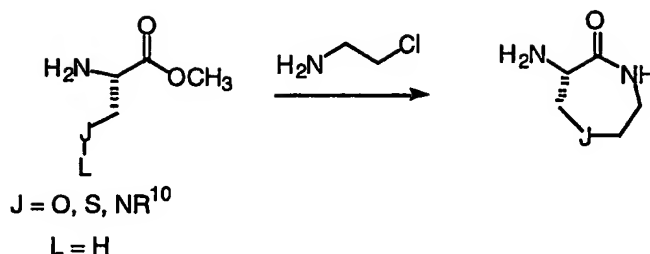
25 Additional methods useful for the preparation of succinate derivatives are known by those skilled in the art. Such references include, McClure and Axt, Bioorganic & Medicinal Chemistry Letters, **8** (1998) 143-146; Jacobson

and Reddy, Tetrahedron Letters, Vol 37, No. 46, 8263-8266 (1996); Pratt et al., SYNLETT, May 1998, p. 531.



A variety of compounds of Formula (I) can be prepared by methods described in Scheme 4. The protected α -amine **3** of the α -amino- ϵ -caprolactam can be prepared by methods well known in the literature for amino protecting groups as discussed in Theodora W. Greene's book "Protective Groups in Organic Synthesis", such as *N*-Boc using di-*t*-butyldicarbonate in an appropriate solvent like DMSO. A sulfur atom can be introduced into the ring providing L- α -amino- β -thio- ϵ -caprolactam according to the procedure in S. A. Ahmed et al, FEBS Letters, (1984), vol. 174, pages 76-9 (Scheme 3). One skilled in the art can extend this methodology to the synthesis of β -amino and oxygen containing rings by analogy. The sulfur-containing molecules can also be oxidized to the sulfoxide and sulfone by methods known to one skilled in the art.

Scheme 3

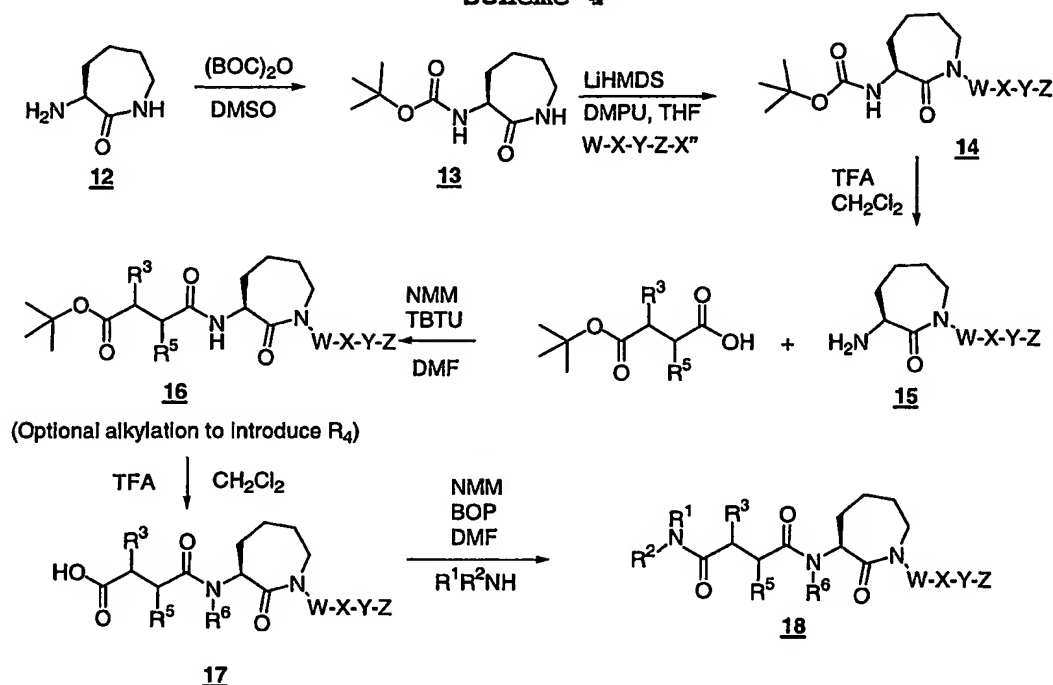


The lactam nitrogen of compound 13 can be alkylated by
 5 generating the anion with bases such as LDA, lithium
 bis(trimethylsilyl)amide or sodium hydride in solvents like
 THF, with or without cosolvents such as DMPU or HMPA and
 reacting this with a variety of groups containing leaving
 groups (X") like bromide, iodide, mesylate or tosylate.
 10 Alkylating agents such as α -bromo amides, ketones and acids
 can be prepared by a number of literature methods including
 halogenation of amino acids by diazotization or are
 commercially available. Other suitable alkylating agents
 such as alkyl, allylic and benzylic halides can be formed
 15 form a variety of precursors such as free-radical addition
 of halides or activation of alcohols, and other chemistries
 known to those skilled in the art. For discussion of these
 types of reactions, see Carey, F.A. and Sundberg, R. J.,
 Advanced Organic Chemistry, Part A, New York: Plenum Press,
 20 1990, pages 304-305, 342-347, 695-698.

The N-Boc protecting group can be removed by any
 number of methods well known in the literature like TFA in
 methylene chloride to give the compound 15. The amine 15
 can be coupled to an appropriately substituted carboxylic
 25 acid or acid chloride by methods well described in the
 literature for making amide bonds, like TBTU in DMF with a
 base like NMM to give the elaborated compound 16.
 Compounds 16 can be alkylated using standard bases like
 LDA, NaH, or NaHMDS to deprotonate the amide followed by
 30 addition of an alkylating agent with an appropriate leaving
 group like halide, mesylate, or triflate in an appropriate
 solvent to provide compounds 17 with an R⁶ substituent.

The t-butyl ester is then removed by treatment with TFA in methylene chloride to give the carboxylic acid 17.

Scheme 4



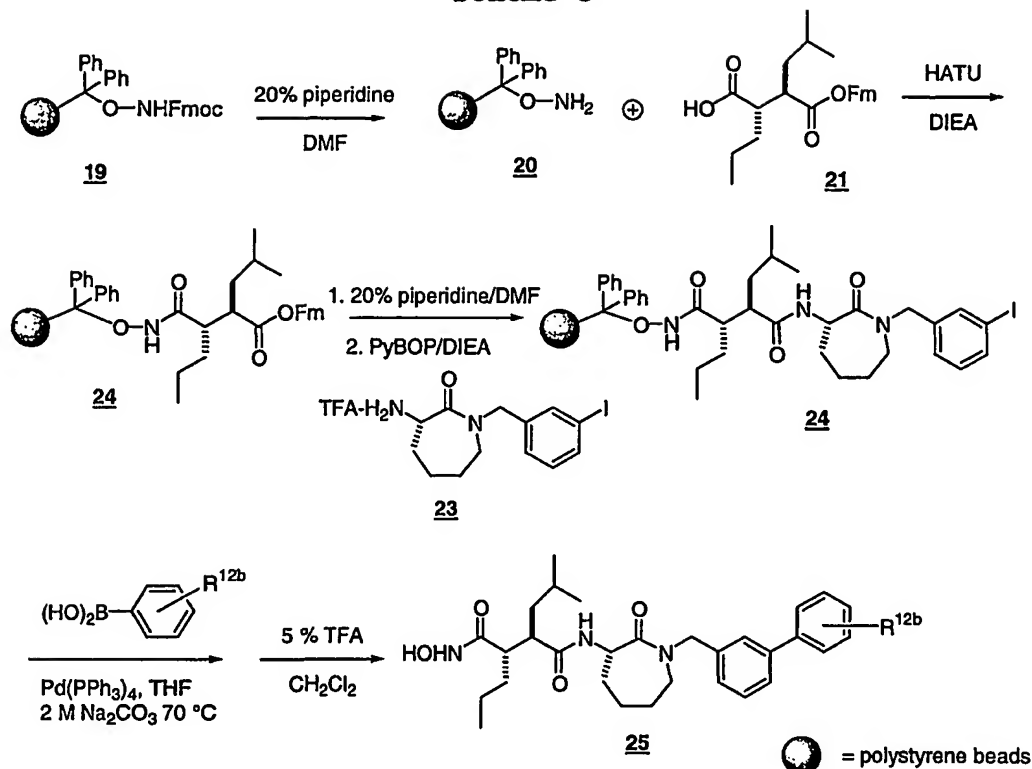
5
 10
 15
 20

The final compounds 18 were prepared by treating the activated carboxylic acid of 17 with an appropriately substituted amine. For instance, activation of the carboxylic acid with HATU (*O*-(7-azabenzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate) or PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate) or other coupling agents known to those skilled in the art allows condensation with ammonia to form primary amides. Similarly, condensation of the activated acid with hydroxylamine hydrochloride provides the hydroxamic acid, or reaction with a primary or secondary amine provides the substituted amine derivative. Activation of the acid with PyBrOP (bromo-tris-pyrrolidino-phosphonium hexafluorophosphate) followed by addition of an alcohol and 4-dimethylaminopyridine allows formation of the ester directly. For additional acylation reactions see for example Carey, F.A. and Sundberg, R. J., Advanced Organic

Chemistry, Part A, New York: Plenum Press, 1990, pages 475-479.

Additional Examples of compounds of Formula (I) can be prepared as shown in Scheme 5. A suitable resin for solid
5 phase synthesis such as Fmoc (Fluorenylmethylcarbonyl)-protected hydroxylamine bound to polystyrene beads can be purchased from Novabiochem, Inc. Deprotection of the Fmoc group under standard conditions using 20% piperidine in DMF provides trityl-linked hydroxylamine resin. Coupling of a
10 fluorenylmethyl-protected succinic acid derivative such as 20 with a coupling agent such as HATU in a suitable solvent like DMF or N-methylpyrrolidinone provides the support-bound hydroxamate 21. The Fluorenylmethyl ester can be removed using 20% piperidine in DMF to provide the free
15 carboxylic acid which can be coupled to amines like the caprolactam 22 (which is available using chemistry outlined in Scheme 4) using PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate) and a suitable base like DIEA in DMF or NMP. The support-bound
20 intermediate 23 can then be elaborated to biaryl structures of the type 24 using typical Suzuki coupling conditions employing a catalyst such as Palladium complexes like tetrakis(triphenylphosphine)-palladium with 2M aqueous sodium carbonate as a base in a suitable solvent like THF
25 or DME and an excess of a boronic acid. The final compounds are liberated from the support employing dilute (5%) trifluoroacetic acid in CH₂Cl₂ and purified by conventional chromatography.

Scheme 5



5 General procedure for solid-phase synthesis according to Scheme 5.

Resin 20 of Scheme 5: Fmoc-protected resin **19** (2.0 g, 0.78 mmol/g, 1.56 mmol) is purchased from Novabiochem and swelled in 20 ml of CH₂Cl₂ for 1 hour. The CH₂Cl₂ is removed and the resin is then treated with 25% v/v piperidine in DMF (8 mL) and allowed to shake slowly for 16 h. The solvent was removed by filtration and the resin was shaken with an additional 8 mL of 25% v/v piperidine in DMF for 2 h at rt. The solvents were removed by filtration, and the resin **20** was rinsed 3 x with 20 mL of DMF, 3 x with 20 mL of methanol, and 3 x with 20 mL of CH₂Cl₂ and dried in vacuo.

Succinate 10 of Scheme 2: Succinate **9** is prepared according to the literature procedure (P. Becket, M. J. Crimmin, M. H. Davis, Z. Spavold, Synlett, (1993), 137-138). Succinate **9** (17.8 g, 66 mmol) is dissolved in 250 mL

of ethyl acetate and placed in a Parr shaker bottle. To the solution is added 890 mg of 5% palladium on carbon, and the bottle is pressurized to 40 psi with hydrogen gas and shaken for 2.5 h at rt. The hydrogen is removed and the
5 palladium catalyst is removed by filtration through a pad of celite. Concentration of the ethyl acetate solution provides 17.5 g (98%) of succinate 10. No further purification is necessary. MS (M-H)⁺ = 271.

Succinate 21 of Scheme 5: Succinate 10 (6.3 g, 23.1
10 mmol) is dissolved in 125 mL of CH₂Cl₂ and 4.8 g (23.3 mmol) of dicyclohexylcarbodiimide is added. The solution is stirred at rt for 30 min and then 4.6 g (23.4 mmol) of 9-fluorene-methanol is added followed by 122 mg (1 mmol) of 4-dimethylaminopyridine. After 5 h of stirring at rt, the
15 reaction solution was diluted with an additional 100 mL of CH₂Cl₂ and filtered through a pad of celite to remove precipitated dicyclohexylurea. The solution was then washed 3 x with 50 mL of a 1N HCl solution, 3 x with 50 mL of a saturated sodium bicarbonate solution, and 2 x with 50
20 mL of brine. The crude product was dried over MgSO₄ and concentrated onto 15 g of silica gel. Chromatography eluting with a gradient of 2.5% to 5% ethyl acetate/hexanes provided 6.4 g (61%) of the diester as an oil. The purified diester (6.4 g 14.2 mmol) is then dissolved in 25
25 mL of CH₂Cl₂, 25 mL of trifluoroacetic acid is added, and the reaction solution is stirred at rt for 2 h. The reaction solution is directly concentrated *in vacuo* to an oil which is then redissolved in 25 mL of toluene and reconcentrated, followed by drying *in vacuo* to provide 6.3
30 g (98%) of the desired succinate 9 as an oil which solidifies on standing. MS (M+Na)⁺ = 471, (M+2Na)⁺ = 439.

Caprolactam 23 of Scheme 5: Boc-caprolactam 14 (5.0 g, 21.9 mmol) is dissolved in 60 mL of THF and chilled to -78°C. To the chilled solution is added 24 mL of a 1.0 M
35 solution of lithium bis(trimethylsilyl)amide in THF, and the solution was brought to 0°C and stirred for 15 min. To the anion solution was added 6.5 g (22 mmol) of 3-iodobenzyl bromide (Aldrich) and the the solution was

allowed to warm to rt and stirred for 18 h. The reaction solution was diluted with 50 mL of water and extracted 3x with ethyl acetate. The combined organic layers were dried over MgSO_4 and concentrated in vacuo. The crude product
5 was purified by chromatography eluting with a gradient of 5-20% ethyl acetate/hexanes to afford 7.0 g (72%) of the title compound as a white solid. MS $(\text{M}+\text{Na})^+ = 467$.

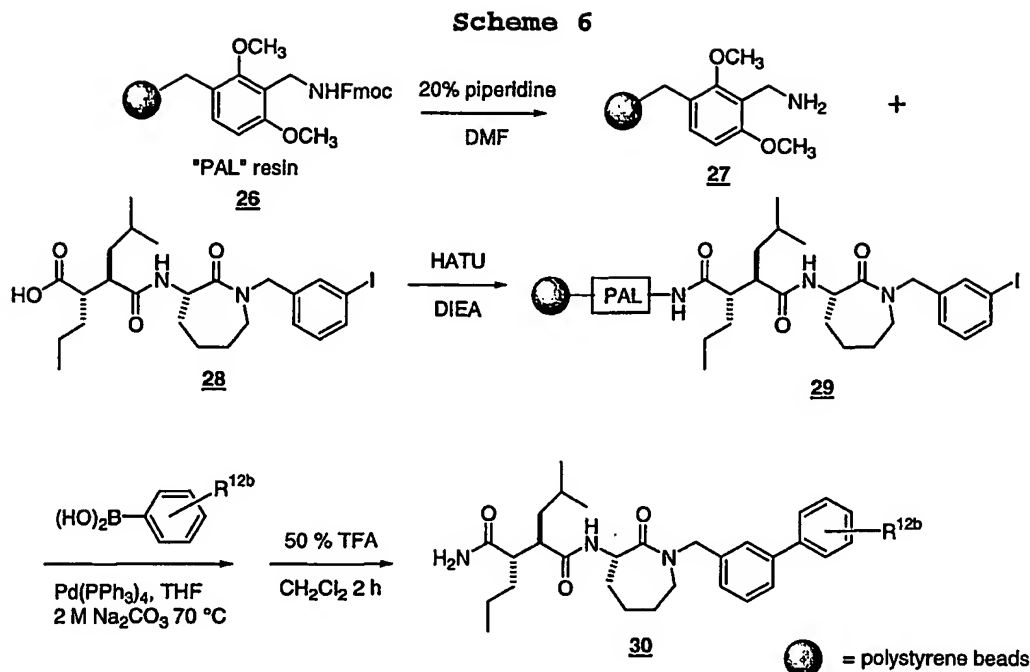
Resin 22 of Scheme 5: Resin 22 (2.0 g, 0.78 mmol/g, 1.56 mmol) was swollen in 3 mL of DMF. In a separate
10 flask, 1.85 g (4.68 mmol) of succinate 21 was dissolved in 3 mL of DMF and 2.5 mL of *N,N*-diisopropylethylamine (14 mmol) was added, followed by 1.81 g (4.68 mmol) of HATU. The solution containing the active ester was added to the slurried resin and the reaction suspension was slowly
15 shaken for 18 h. The resin was then washed 3 x with 20 mL of DMF, 3 x with 20 mL of methanol, and 3 x with 20 mL of CH_2Cl_2 . Loading of the resin was determined by Fmoc quantitation to be 0.25 mmol/g, see Reddy, M. P.; Voelker, P.J. *Int. J. Pept. Protein Res.* 1998, 31, 345-348.

Resin 24 of Scheme 5: Resin 22 (2.0 g, 0.25 mmol/g, 0.5 mmol) was suspended in 10 mL of 25% piperidine in DMF. The suspended resin was shaken for 30 min at rt, and then the resin was washed 3 x with 20 mL of DMF, 3 x with 20 mL of methanol, and 3 x with 20 mL of CH_2Cl_2 . Deprotected
25 resin (1.0 g, 0.25 mmol) was swollen in 2 mL of DMF. To the slurry was added 650 mg (1.25 mmol) of PyBOP and 217 mL (1.25 mmol) of DIEA. Separately, 443 mg (0.97 mmol) of caprolactam 23 was dissolved in 2 mL of DMF and 436 mL (2.5 mmol) of DIEA was added. The caprolactam solution was
30 added to the resin slurry and the resin was mixed for 18 h at rt. The solvents were then removed and the coupling was repeated, with shaking at rt for 6 h. The resin was then washed 3 x with 10 mL of DMF, 3 x with 10 mL of methanol, and 3 x with 10 mL of CH_2Cl_2 .

Products 25 of Scheme 5: A 70 mg (17.5 mmol) portion of resin 24 was suspended in 1 mL of THF in a screw-cap vial. To the slurry was added a boronic acid (0.15 mmol), 150 mL of a 2 M solution of sodium carbonate, and 15 mg (13

mmol) of tetrakis(triphenylphosphine)palladium. The vial was tightly closed and heated to 60°C for 16 h using a dry heater on a shaker table. The solvents were then removed by filtration and the resin was washed 3 x with THF (2 mL), 3 x with methanol (2 mL), 3 x with water, and 3 x with CH₂Cl₂. The resins were then placed in a glass vial and cleaved with 1 mL of 5% trifluoroacetic acid in CH₂Cl₂ for 30 min. The solution was filtered off and the resin was washed with an additional 2 mL of CH₂Cl₂ and the combined filtrates were evaporated to dryness to yield the crude products 25. The products were purified by chromatography eluting with 10-100% ethyl acetate in hexanes to yield 13.0 to 6.0 mg (14-60%) of the final products.

Additional Examples of compounds of Formula (I) can be prepared as shown in Scheme 6. A suitable resin for solid phase synthesis such as Fmoc (Fluorenylmethylcarbonyl)-protected peptide amide linker (PAL)-derivatized polystyrene beads can be purchased from Perkin Elmer Biosystems, Inc. Deprotection of the Fmoc group under standard conditions using 20% piperidine in DMF provides the free benzylamine. Coupling of a succinic acid derivative such as 28 (which is available using chemistry outlined in Scheme 4) with a coupling agent such as HATU in a suitable solvent like DMF or N-methylpyrrolidinone provides the support-bound amide 29. The support-bound intermediate 29 can then be elaborated to biaryl structures of the type 24 using typical Suzuki coupling conditions employing a catalyst such as Palladium complexes like tetrakis(triphenylphosphine)-palladium with 2M aqueous sodium carbonate as a base in a suitable solvent like THF or DME and an excess of a boronic acid. The final compounds are liberated from the support employing 50% trifluoroacetic acid in CH₂Cl₂ and can be purified by conventional chromatography or preparative HPLC.



General procedure for solid-phase synthesis according to Scheme 6

Resin 27 of Scheme 6: Fmoc-protected PAL resin 26 (0.80 g, 0.50 mmol/g, 0.40 mmol) is purchased from Advanced Chemtech and swelled in 20 mL of CH_2Cl_2 for 1 hour. The CH_2Cl_2 is removed and the resin is then treated with 25% v/v piperidine in DMF (6 mL) and allowed to shake slowly for 1 h. The solvents were removed by filtration, and the resin 27 was rinsed 3 x with 20 mL of DMF, 3 x with 20 mL of methanol, and 3 x with 20 mL of CH_2Cl_2 . and dried in vacuo.

Acid 28 of Scheme 6: To a solution of 0.100 g (367 μ mol) of succinate 10 dissolved in 2.0 mL of dry DMF was added 0.120 mL (1.10 mmol) of N-methylmorpholine. A second solution containing 0.139 g (0.403 mmol) of caprolactam 23 of Scheme 5 dissolved in 2.0 mL of DMF was then added. To the mixed solution was added 229 mg (0.440 mmol) of PyBop and the reaction solution was stirred for 16 h at rt. The reaction solution was diluted with water (20 mL) and extracted 3 x with 100 mL of ethyl acetate. The combined organic layers were dried with Na_2SO_4 and concentrated

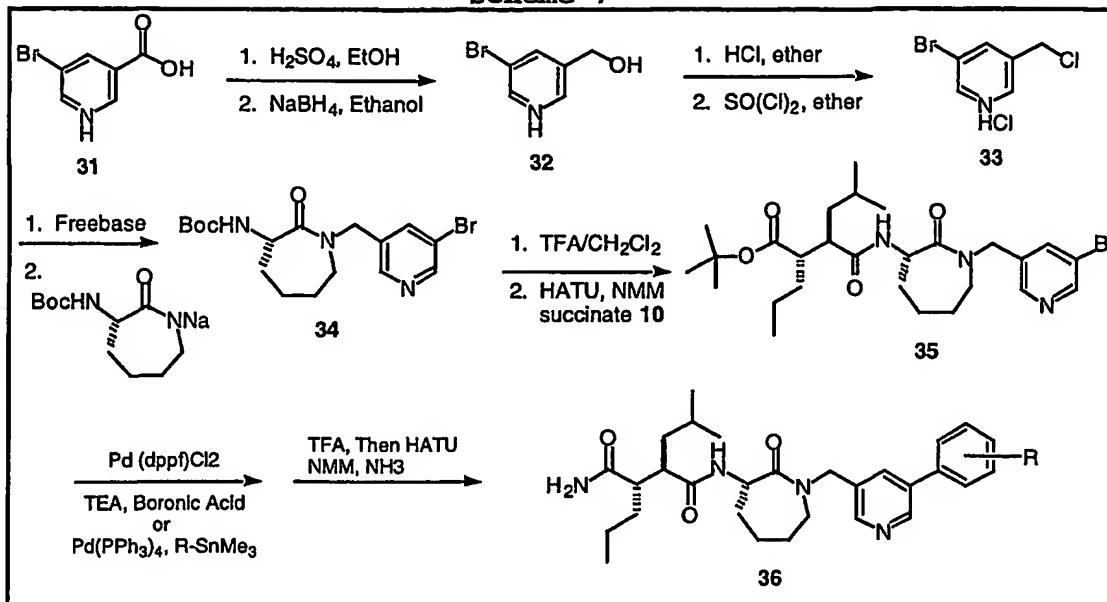
under reduced pressure. The resulting oil was purified by chromatography eluting with a gradient of 5-20% ethyl acetate in hexanes to provide 0.195 g (0.360 mmol, 98%) of the *tert*-butyl ester of Acid 28 (MS $M+Na=621$). The
5 purified ester (0.195 g, 0.360 mmol) was dissolved in 10 mL of 25% trifluoroacetic acid in CH_2Cl_2 and stirred for 2 h at rt. The solvents were removed under reduced pressure and the acid was redissolved in 5 mL of toluene and reconcentrated 2 x to remove residual TFA. The crude acid
10 was found to be pure by 1H NMR and was used in Scheme 6 without further purification.

Resin 29 of Scheme 6. Resin 27 (800 mg, 0.40 mmol) was solvated in 4.0 mL of dry DMF and 0.63 mL (3.6 mmol) of diisopropylethylamine was added followed by a
15 solution of Acid 28 dissolved in 4 mL of DMF. To the slurry was then added 0.465 g (1.2 mmol) of HATU and the slurry was shaken for 26 h at rt. The solvents were removed by filtration, and the resin 29 was rinsed 3 x with 20 mL of DMF, 3 x with 20 mL of methanol, and 3 x with 20 mL of
20 CH_2Cl_2 . and dried in vacuo.

Products 30 of Scheme 6: A 75 mg (0.38 mmol/g, 28.8 μ mol) portion of resin 24 was suspended in 1 mL of THF in a screw-cap vial. To the slurry was added a boronic acid (0.33 mmol), 150 mL of a 2 M solution of sodium carbonate,
25 and 15 mg (13 mmol) of tetrakis(triphenylphosphine)palladium. The vial was tightly closed and heated to 60°C for 16 h using a dry heater on a shaker table. The solvents were then removed by filtration and the resin was washed 3 x with THF (2 mL),
30 3 x with methanol (2 mL), 3 x with water, and 3 x with CH_2Cl_2 . The resins were then placed in a glass vial and cleaved with 1 mL of 5% trifluoroacetic acid in CH_2Cl_2 for 2 h. The solution was filtered off and the resin was washed with an additional 2 mL of CH_2Cl_2 and the combined filtrates
35 were evaporated to dryness to yield the crude products 25. The products were purified by chromatography eluting with 10-100% ethyl acetate in hexanes to yield 0.5 to 2.0 mg (14-60%) of the final products.

The internal phenyl ring can be exchanged for a pyridine ring using chemistry outlined in Scheme 7. The chloromethyl pyridine 33 is prepared using a known procedure reported in Nutaitis, Charles F.; Ledebor, Mark W. Org. Prep. Proced. Int. (1992), 24(2), 143-6 Incorporated herein by reference. After freebasing the pyridine, alkylation with the Boc-caprolactam provides pyridine intermediate 34, which can be elaborated to the protected amide 35 with succinate 10. Substitution can then be introduced using Suzuki methodology employing a palladium source such as tetrakis(triphenylphosphine) palladium(0) or bis(diphenylphosphinoferrocene) palladium(II) dichloride and a suitable base such as sodium carbonate or triethylamine in a solvent such as THF or toluene containing 10% methanol. Stille chemistry is also possible using a suitable palladium source such as tetrakis(triphenylphosphine)palladium(0) and an aryl or vinyl tin derivative in a solvent such as benzene, toluene, or xylenes. The tert-butyl ester is then deprotected under standard acidic conditions using trifluoroacetic acid and the amide is formed under standard conditions to provide products 36.

Scheme 7



5 General procedure for synthesis according to Scheme 7

The chloromethyl pyridine HCl salt **33** is prepared using a known procedure reported in Nutaitis, Charles F.; Ledebor, Mark W. Org. Prep. Proced. Int. (1992), 24(2), 143-6.

- 10 Caprolactam **34**: Pyridine HCl salt **33** (2.0 g, 8.3 mmol) is dissolved in 50 mL of a saturated NaHCO_3 solution and the solution is extracted with 30 mL of CH_2Cl_2 3 x followed by concentration of the organic layers to provide the free base. Separately, 1.8 g (7.8 mmol) of caprolactam
- 15 **13** is dissolved in 40 mL of dry THF and chilled to -78°C . To the solution was added 8.7 mL of a 1M solution of sodium bis(trimethylsilyl) amide. The solution was brought to 0°C and stirred for 30 min. To the resultant anion was added a solution of 1.7 g (8.3 mmol) of pyridine **33** free base
- 20 dissolved in 40 mL of THF. The resulting reaction solution was stirred at rt for 18 h and then heated to 50°C and stirred an additional 3 h. The reaction solution was allowed to cool and then 50 mL of water was added and the aqueous layer was extracted 2 x with 100 mL of ethyl
- 25 acetate. The combined organic layers were dried and

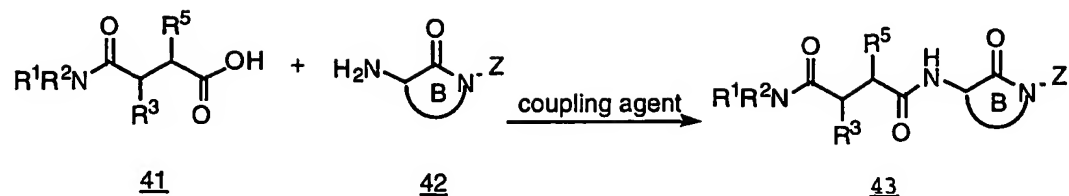
concentrated under reduced pressure to provide the crude product which was purified by chromatography eluting with 20 to 100% ethyl acetate in hexanes to provide 1.5 g (51%) of caprolactam 34 as an oil.

5 Amide 35: Caprolactam 34 (0.40 g, 1.0 mmol) is dissolved in 20 mL of 50% trifluoroacetic acid in CH₂Cl₂ and stirred at rt for 30 min. The solvents were then removed under reduced pressure and the resulting oil was redissolved in 5 mL of toluene and reconcentrated to remove
10 residual TFA. Separately, 0.270 g (1.0 mmol) of succinate 10 was dissolved in 5.0 mL of dry DMF and 0.44 mL (4 mmol) of *N*-methyldmorpholine was added followed by 0.50 g (1.3 mmol) of HATU and the resulting solution was stirred at rt for 30 min. The crude deprotected caprolactam from above
15 was dissolved in 5.0 mL of dry DMF and added to the succinate solution and the resulting solution was heated to 50 °C and stirred for 2 days. The solution was then diluted with 20 mL of water and extracted with 3 50 mL portions of ethyl acetate. The combined organic layers
20 were dried and concentrated under reduced pressure to provide an oil which was purified by chromatography eluting with 20 to 50% ethyl acetate in hexanes to provide 0.40 g (70%) of the Amide 35.

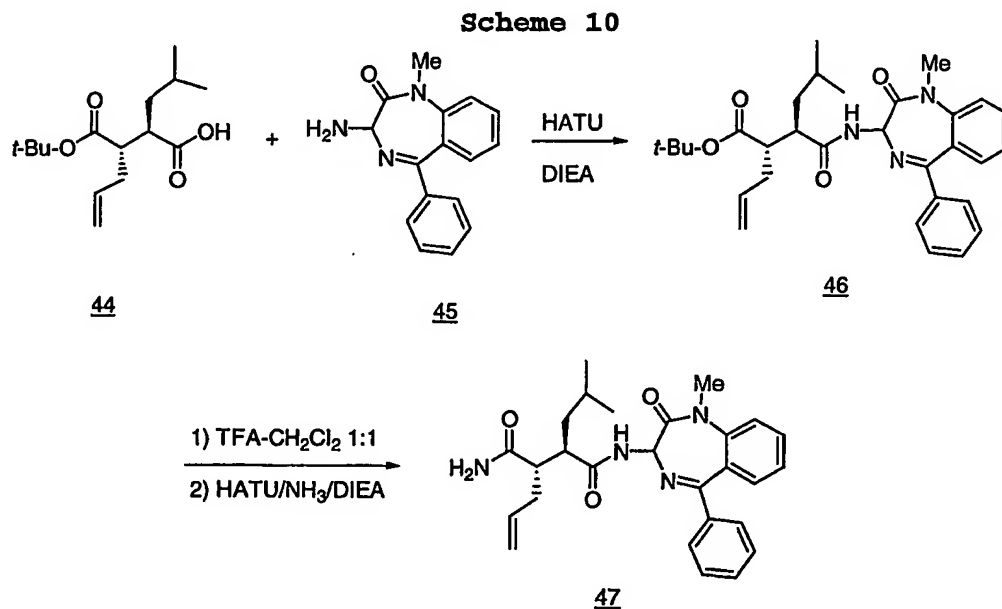
25 The compounds of Formula (I) of the present invention can also be prepared from aminolactam 42 and succinic acid derivatives 41 using amide bond syntheses known in the art, including methods commonly used in peptide syntheses, such as HATU, TBTU, BOP, pyBOP, EDC, CDI, DCC,
30 hydroxysuccinimide, mixed carboxylic anhydride, and phenyl ester mediated couplings, as illustrated in Scheme 9 for the synthesis of aminolactam 43, an embodiment of the present invention.

35

Scheme 9



Depending on the structure of the final product, it is appreciated by those skilled in the art that protecting groups or precursor functionality convertible to the desired groups may be desirable. Protecting groups and their use in synthesis are described in Green and Wuts, *Protective Groups in Organic Synthesis*, (Wiley 1991). The use of protecting groups is further illustrated in Scheme 10, in which the succinate half-ester **44** (Becket et al., Synlett 1993, 137-138) is coupled to the aminobenzodiazepine **45** (Sherrill and Sugg, J. Org. Chem. 1995, 60, 730-734; Bock et al., J. Med. Chem., 1993, 36, 4276-4292) to give ester **46**, followed by conversion of the ester group to the primary amide **47**.



20

Methods for the synthesis of lactams as contemplated by the present invention in lactam ring B in Formula (I),

including amino benzodiazepines, are known in the art and are disclosed in a number of references including PCT publication number WO 98/28268, which is hereby incorporated by reference. Additional references include

5 Bock, et al, J. Org. Chem., 1987, 52, 3232-3239 and Sherrill et al, J. Org. Chem., 1995, 60, 730-734; Walsh, D. A., Synthesis, September 1980, p.677.

Examples

10 Chemical abbreviations used in the Examples are defined as follows: "DMPU" for 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidone, "TBTU" for O-(1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate, and

15 "BOP" for benzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate. It is understood that one skilled in the art can discern compounds used in the synthesis of Examples of the invention may be referred to by structure and number. For example, Resin 20 refers to the resin of structure 20 in

20 Scheme 5; succinate 9 refers to the structure 9 found in Scheme 2 which is a succinate compound.

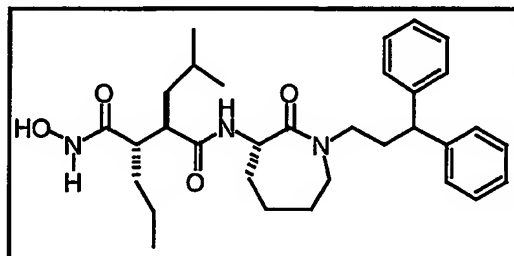
"HPLC" is an abbreviation used herein for high pressure liquid chromatography. Reverse-phase HPLC was carried out using a Vydac C-18 column with gradient elution

25 from 10% to 100 % buffer B in buffer A (buffer A: water containing 0.1% trifluoroacetic acid, buffer B: 10% water, 90% acetonitrile containing 0.1% trifluoroacetic acid).

Example 1

(2R,3S) N1-[(3S)-hexahydro-1-(3,3-diphenylpropyl)-2-oxo-1H-azepin-3-yl]-N4-(hydroxy)-2-(2-methylpropyl)-3-(propyl)-butanediamide.

5



Step (1a): Di-*tert*-butyldicarbonate (10.2 g, 46.7 mmol) was added portion wise to a solution of L-(-)- α -amino- ϵ -caprolactam (5.0 g, 39.0 mmol) in dimethyl sulfoxide (30 mL). After 5 h at rt, the reaction was partitioned between water (100 mL) and ethyl acetate. The combined organic extracts were washed successively with 1 M HCl (50 mL), brine, and dried (MgSO₄) and concentrated *in vacuo*. The residue was recrystallized in 1:1 v/v ether-hexanes, two crops yielded the desired product (6.26 g, 70%) as white solid. MS (M+H-BOC)⁺ = 129.

Step (1b): Triphenylphosphine (3.0 g, 11.4 mmol) and carbon tetrabromide (3.75 g, 11.7 mmol) were added successively to a cooled (0°C) solution of 3,3-biphenyl-1-propanol (1.5 mL, 7.5 mmol) in dichloromethane (20 mL). After 1.5 hours at rt, the mixture was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (hexanes) to give the desired product (1.93 g, 93% yield) as a clear oil. MS (M-BrC₂H₄)⁺ = 167

Step (1c): A 1.0 M tetrahydrofuran solution of lithium bis(trimethylsilyl)amide (1.3 mL) was added over 15 minutes to compound of Step (1a) (0.29 g, 1.27 mmol) in tetrahydrofuran (3 mL) and DMPU (2 mL) at -78°C. The iodo compound prepared from compound (1b) (0.85 g, 3.09 mmol) by typical Finkelstein methodology, in tetrahydrofuran (4 mL) was added and the reaction was allowed to warm to rt

- slowly. This was stirred for 10 hours at ambient temperature, partitioned between water and ethyl acetate. The combined organic extracts were washed successively with water (20 mL), brine (20 mL), and dried (MgSO_4) and concentrated *in vacuo*. The resulting residue was purified by silica gel column (ethyl acetate:hexanes, 5:95 then ethyl acetate:hexanes, 15:85) to give the desired product (0.16 g, 30%). MS (M-Ot-Bu)⁺ = 349.
- 10 Step (1d): Trifluoroacetic acid (3 mL) was added to a solution of compound of Step (1c) (0.16 mg, 0.38 mmol) in dichloromethane (9 mL). After 2 h at rt, the solvent was removed *in vacuo*. The residual trifluoroacetic acid was removed by azeotrope with dichloromethane (50 mL), toluene (50 mL), and dichloromethane (50 mL) successively to give the desired product (0.17 g, 99%) as a yellow oil. MS (M+H)⁺ = 323.
- 20 Step (1e): 4-Methylmorpholine (0.6 mL, 5.46 mmol) and TBTU (0.11 g, 0.34 mmol) were added to a solution of succinate acid (P. Becket, M. J. Crimmin, M. H. Davis, Z. Spavold, Synlett, (1993), 137-138) (0.085 g, 0.31 mmol) in *N,N*-dimethylformamide (3 mL). After 30 minutes at rt, the compound from step (1d) (0.17 g, 0.39 mmol) was added to the mixture. The reaction was stirred for 16 h at rt, then partitioned between 1 M HCl (20 mL) and ethyl acetate. The combined organic extracts were washed successively with saturated aqueous sodium bicarbonate (20 mL), water (20 mL), brine (20 mL), dried (MgSO_4) and concentrated *in vacuo*. The residue was purified by silica gel chromatography (ethyl acetate:hexanes, 7:93 gradient to ethyl acetate:hexanes 25:75) to give the desired product (120 mg, 67%) as a clear oil. MS ($\text{M+NH}_4\text{-Ot-Bu}$)⁺ = 521.
- 35 Step (1f): Trifluoroacetic acid (3 mL) was added to a solution of compound of Step (1e) (120 mg, 0.21 mmol) in dichloromethane (9 mL). After 3 hours at rt, the mixture was concentrated *in vacuo*. The residual trifluoroacetic

acid was removed by azeotrope with toluene (1 X 50 mL) and dichloromethane (1 X 50 mL). The residue was triturated with Et₂O:Hexanes 95:5, to give the desired product (75 mg, 70%) as a white solid. MS (M-H)⁻ = 519.

5

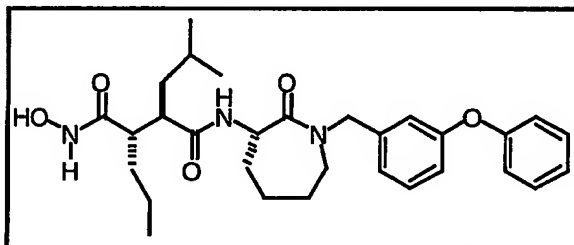
Step (1g): 4-Methylmorpholine (0.05 mL, 0.45 mmoles) and BOP (73 mg, 0.17 mmoles) were added to a solution of compound of Step (1f) (60 mg, 0.12 mmoles) in *N,N*-dimethylformamide (2 mL). Hydroxylamine (33 mg, 0.47
10 mmoles) was added to the mixture, the reaction was stirred for 16 h at rt, was concentrated *in vacuo*, was acidified with trifluoroacetic acid, then purified by reverse phase HPLC on a Vydac C-18 column, to give the desired hydroxamic acid as a white solid (45 mg, 75%). MS (M-H)⁻ = 534.

15

Example 2

(2R,3S) N1-[(3S)-hexahydro-1-(3-phenoxybenzyl)-2-oxo-1H-azepin-3-yl]-N4-(hydroxy)-2-(2-methylpropyl)-3-(propyl)-
20 butanediamide.

20



Step (2a): Triphenylphosphine (3.40 g, 13.0 mmoles) and carbontetrabromide (4.20 g, 13.0 mmoles) were added successively to a solution of *m*-phenoxybenzyl alcohol (1.5
25 mL, 8.6 mmoles). After 4 h at rt the mixture was concentrated and was purified by silica gel column (hexanes, then ethyl acetate:hexanes, 5:95) to give the desired bromide (1.3 g, 57%) as a yellow oil. MS (M-Br)⁺ = 183.

30

Step (2b): A 1 M solution of lithium bis(trimethylsilyl)amide was added dropwise to a solution of compound of Step (1a) (0.3 g, 1.31 mmoles) in

tetrahydrofuran (5 mL) at -78°C . After 30 minutes a solution of compound of Step (2a) (0.43 g, 1.63 mmol) in tetrahydrofuran (4 mL) was added to the mixture dropwise. The reaction was allowed to come to ambient temperature, stirred for 16 h, then partitioned between water and ethyl acetate. The combined organic extracts were washed successively with water (20 mL), brine (20 mL), dried (MgSO_4) and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (ethyl acetate:hexanes, 5:95 then ethyl acetate:hexanes, 15:85) to give the desired product (360 mg, 67%) as a clear oil. MS (M-Ot-Bu) $^{+}$ = 337.

Step (2c): Trifluoroacetic acid (5 mL) was added to a solution of compound of Step (2b) in dichloromethane (15 mL). After 3 h at rt the solution was concentrated *in vacuo*. The residual trifluoroacetic acid was removed from residue by azeotrope with toluene (50 mL) then dichloromethane (30 mL) to yield the desired amine (390 mg, 99%) as a clear oil. MS (M+H) $^{+}$ = 311.

Step (2d): Following a procedure analogous to the preparation of Step (1e), but using the compound from Step (2c) (390 mg, 0.88 mmol) the amide was prepared. The crude compound was purified by silica gel chromatography to give the desired product (0.38 g, 92%) as a yellow oil. MS (M-Ot-Bu) $^{+}$ = 491.

Step (2e): Following a procedure analogous to the preparation of step (1f), but using the compound from Step (2d) (380 mg, 0.67 mmol), the carboxylic acid was prepared. The product was precipitated from ethyl ether with hexanes, to give the desired acid (227 mg, 66%) as a white solid. MS (M-H) $^{-}$ = 507.

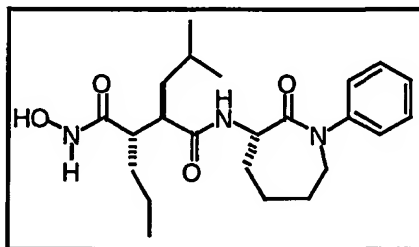
Step (2f): Following a procedure analogous to the preparation of compound of Step (1g), but using the compound from step (2e) (150 mg, 0.29 mmol) the title

compound was prepared. The crude was purified by reverse phase HPLC on a Vydac C-18 column to give the desired product (90 mg, 58%) as a white solid. MS (M-H)⁻ = 522.

5

Example 3

(2R,3S) N1-[(3S)-hexahydro-1-(phenyl)-2-oxo-1H-azepin-3-yl]-N4-(hydroxy)-2-(2-methylpropyl)-3-(propyl)-butanediamide.



10

Step (3a): Triethylamine (1.5 mL, 10.8 mmol), copper (II) acetate (0.95 g, 5.2 mmol) and phenylboric acid (1.6 g, 13.1 mmol) were added successively to a solution of compound of Step (1a) (1.0 g, 4.4 mmol) in dichloromethane (20 mL). After 2.5 h at rt, more phenylboric acid (0.5 g, 4.1 mmol) was added to the mixture. After an additional 3 hours at rt more phenylboric acid (0.5 g, 4.1 mmol) was added to the mixture. After 65 h at rt, the mixture was filtered over celite. The filtrate was concentrated in vacuo, and the residue was purified by silica gel chromatography (ethyl acetate:hexanes, 5:95 then 15:85) to give the desired product (250 mg, 19%). MS (M-Ot-Bu)⁺ = 231.

25 Step (3b): Following a procedure analogous to the preparation of compound of Step (2c), but using compound of Step (3a) (250 mg, 0.82 mmol), the amine (300 mg, 99%) was prepared as a yellow oil. MS (M+H)⁺ = 205.

30 Step (3c): Following a procedure analogous to the preparation of compound of Step (1e), but using compound from Step (3b) (0.3g, 0.94 mmol), the amide was prepared. The residue was purified by silica gel chromatography

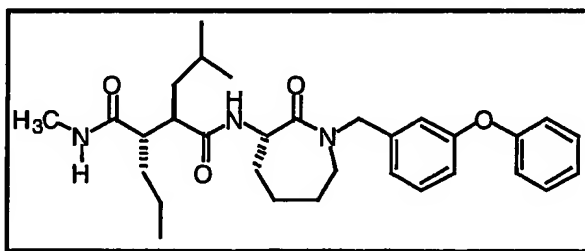
(ethyl acetate:hexanes, 5:95 to 20:80 in 5% increments, 500 mL each ratio) to give the desired product (210 mg, 60%) as a clear oil. MS (M+H-t-Bu)⁺ = 403.

5 Step (3d): Following a procedure analogous to the preparation of compound of Step (1f), but using compound from sStep (3c) (200 mg, 0.44 mmol) the acid was prepared. The crude oil was triturated with ether:hexanes 1:1 to give the desired acid (114 mg, 65%) as a white
10 solid. MS (M-OH)⁺ = 385.

Step (3e): Following a procedure analogous to the preparation of compound of Step (1g), but using compound from Step (3d) (82 mg, 0.20 mmol) the title compound was
15 prepared. The crude product was purified by reverse phase HPLC on a Vydac C-18 column to give the desired product (80 mg, 94%). MS (M-H)⁻ = 416.

Example 4

20 (2R,3S) N1-[(3S)-hexahydro-1-(3-phenoxybenzyl)-2-oxo-1H-azepin-3-yl]-N4-(methyl)-2-(2-methylpropyl)-3-(propyl)-butanediamide.

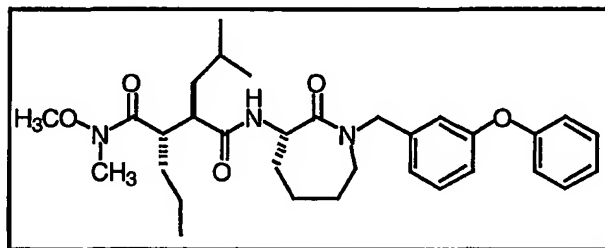


25 Following a procedure analogous to the preparation of Example 3, compound of Step (2e) (100 mg, 0.20 mmol) was treated with HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate) (114 mg, 0.30 mmol) and N-methyl morpholine (66 mL, 0.6 mmol) in 2 mL of DMF
30 for 15 min at rt. A solution of 2.0 M methylamine in THF (0.2 mL, 0.4 mmol) was added and the reaction solution was stirred for 1 h at rt. The reaction solution was diluted with 1N HCl (5 mL) and extracted 3 x with 10 mL of ethyl

acetate. The combined organic layers were washed with a saturated sodium bicarbonate solution (5 mL) and brine (5 mL), dried over magnesium sulfate, and concentrated in vacuo to provide the crude amide. Purification by reverse phase HPLC on a Vydac-18 column provided the desired amide (30 mg, 30%). MS (M+Na)⁺ = 544.

Example 5

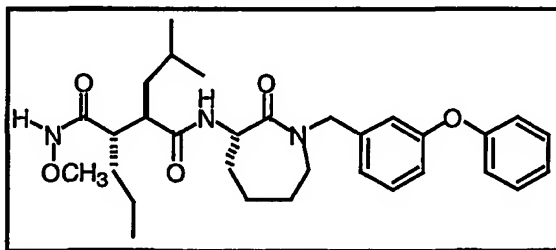
(2R,3S) N1-[(3S)-hexahydro-1-(3-phenoxybenzyl)-2-oxo-1H-azepin-3-yl]-N4-(methoxy)-N4-(methyl)-2-(2-methylpropyl)-3-(propyl)-butanediamide.



Following a procedure analogous to the preparation of Example 4, compound of Step (2e) (100 mg, 0.20 mmol) was activated and condensed with *N,O*-dimethylhydroxylamine hydrochloride (40 mg, 0.40 mmol). Purification by reverse phase HPLC on a Vydac-18 column provided the desired amide (30 mg, 30%). MS (M+Na)⁺ = 574.

Example 6

(2R,3S) N1-[(3S)-hexahydro-1-(3-phenoxybenzyl)-2-oxo-1H-azepin-3-yl]-N4-(methoxy)-2-(2-methylpropyl)-3-(propyl)-butanediamide.

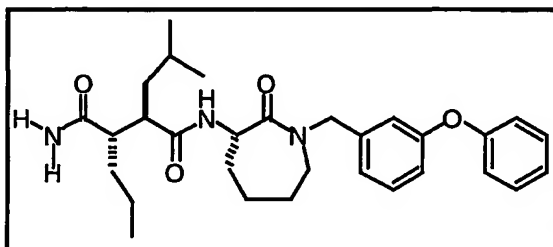


Following a procedure analogous to the preparation of Example 4, compound of Step (2e) (100 mg, 0.20 mmol) was activated and condensed with *O*-methylhydroxylamine hydrochloride (40 mg, 0.40 mmol). Purification by reverse

phase HPLC on a Vydac-18 column provided the desired amide (30 mg, 30%). MS (M+Na)⁺ = 560.

Example 7

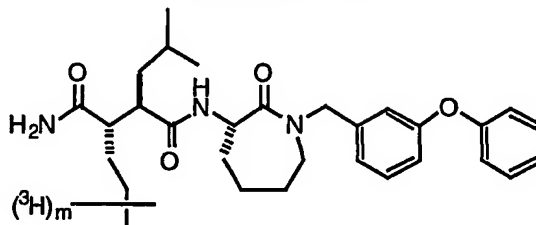
- 5 (2R,3S) N1-[(3S)-hexahydro-1-(3-phenoxybenzyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanedi-
amide.



- Following a procedure analogous to the preparation of
10 Example 4, compound of Step (2e) (100 mg, 0.20 mmol) was
activated and condensed with a 2.0 M solution of ammonia in
dioxane (0.2 mL, 0.4 mmol). Purification by reverse phase
HPLC on a Vydac-18 column provided the desired amide (30
mg, 30%). MS (M+Na)⁺ = 530.

15

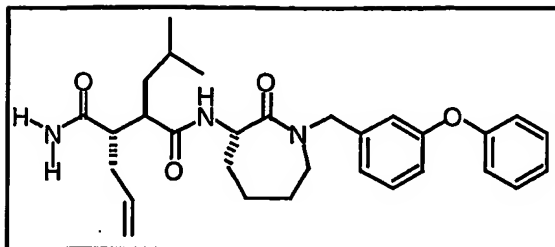
Example 7T



- Example 7T was synthesized by reducing the double bond
present in the compound of Example 8. Thus, the compound
20 of Example 8 was dissolved in tetrahydrofuran and
hydrogenated using tritium gas, by methods known to one
skilled in the art organic synthesis. Purification by
reverse phase HPLC on a Vydac-18 column provided the
desired tritiated amide Example 7T wherein m is
25 approximately 2.

Example 8

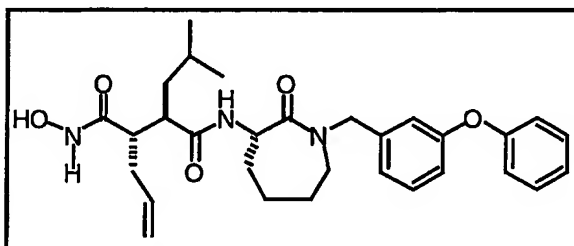
(2R,3S) N1-[(3S)-hexahydro-1-(3-phenoxybenzyl)-2-oxo-1H-
azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanedi-
amide.



Example 8 was synthesized following a procedure analogous to the preparation of Example 7, but using succinate 9 (Scheme 2). The compound was purified by chromatography eluting with 5% methanol in CH₂Cl₂ to afford approx. 500 mg of Example 8. MS (M+Na)⁺ = 528.

Example 9

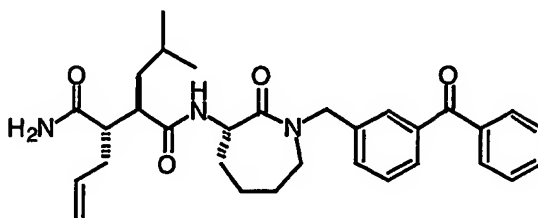
(2R,3S) N1-[(3S)-hexahydro-1-(3-phenoxybenzyl)-2-oxo-1H-azepin-3-yl]-N4-(hydroxy)-2-(2-methylpropyl)-3-(allyl)-butanediamide.



Example 9 was synthesized following a procedure analogous to the preparation of Example 2, but using succinate 9 (Scheme 2). Purification by reverse phase HPLC on a Vydac-18 column provided 150 mg of Example (9). MS (M+Na)⁺ = 544.

Example 10

(2R,3S) N1-[(3S)-hexahydro-1-(benzophenon-3-yl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(allyl)-butanediamide.



(Step 10-a): **3-Bromomethylbenzophenone**. A solution of 3-methylbenzophenone (20 g, 102 mmol) dissolved in 40 mL of 1,2-dibromoethane was heated to reflux. Over a period of about 3 hours a solution of 105 mmol of bromine dissolved in 6 mL of 1,2-dibromoethane was added to the refluxing solution. After the addition was complete the solution was allowed to cool to rt and diluted with 100 mL of dichloromethane. The organic layer was extracted with 1 x 25 mL of 1 N HCl, 2 x 15 mL of NaHCO₃ Solution, and 2 x 25 ML of brine. The organic layers were dried over magnesium sulfate and concentrated *in vacuo*. The residue was then distilled to afford the product, 16.5 g (60 %) as an oil that solidified upon standing, b.p. 160°C at 300 mTorr. ¹H NMR analysis shows that the product contains approximately 7% of the dibromide.

Step (10-b): **3-(1,1-dimethylethylcarbomethoxy-N-(benzophenone-3-yl-methyl)caprolactam**. Diisopropylamine (4.2 mL, 30 mmol) was dissolved in 25 mL of THF and chilled to -78°C. To the solution was added 10 mL of 2.5M n-butyllithium in hexanes and the solution was warmed to 0°C and allowed to stir for 10 min. A solution of Boc-protected aminocaprolactam 1a (5.0 grams, 22 mmol) dissolved in 25 mL of THF was then added and the reaction solution was stirred for 1 h at 0°C. Solid 3-bromomethylbenzophenone was then added and the reaction solution was allowed to warm to rt and stir overnight. The reaction solution was diluted with water and extracted into ethyl acetate (100 mL). The organic layer was rinsed with 2x 25 mL of 1 N HCl, 2 x 25 mL of saturated NaHCO₃ and 2 x 25 mL of brine, dried over magnesium sulfate, and dried *in vacuo*. Chromatography eluting with a gradient of 30% to 40% ethyl acetate in hexanes afforded the pure benzophenone-substituted caprolactam derivative (7.4 g, 80%). MS (M+Na)⁺ = 445.

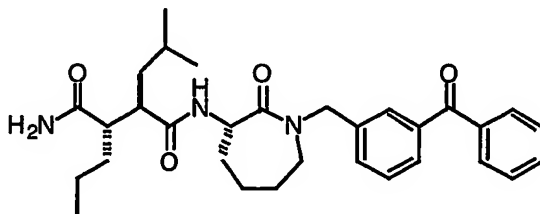
The title compound, Example 10, was synthesized in a manner analagous to the synthesis of the compound of Example 8 using succinate 9 and the benzophenone-

substituted caprolactam derivative of the previous step. The compound was purified by crystallization from ethyl acetate to afford 0.26 g of crystals. MS (M+Na)⁺ = 540.

5

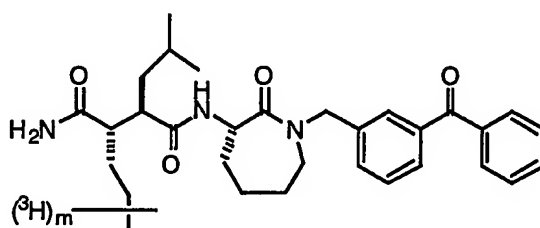
Example 11

(2R,3S) N1-[(3S)-hexahydro-1-(benzophenon-3-yl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.



10

The compound of Example 11 was synthesized in a manner analogous to the synthesis of the compound of Example 8 using succinate 10 and the benzophenone-substituted caprolactam derivative of Step (10-b). The compound was purified by crystallization from ethyl acetate to afford 0.25 g of crystals. MS (M+Na)⁺ = 542.

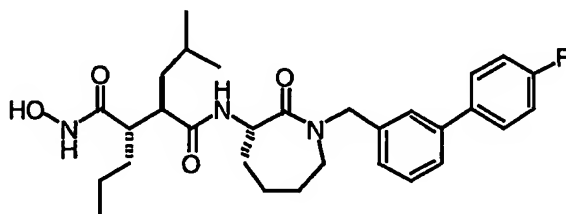
Example 11T

20

Example 11T was synthesized by reducing the double bond present in the compound of Example 10. Thus, the compound of Example 11T was dissolved in tetrahydrofuran and hydrogenated using tritium gas, by methods known to one skilled in the art organic synthesis. Purification by reverse phase HPLC on a Vydac-18 column provided the desired tritiated amide Example 11T wherein m is approximately 2.

Example 13

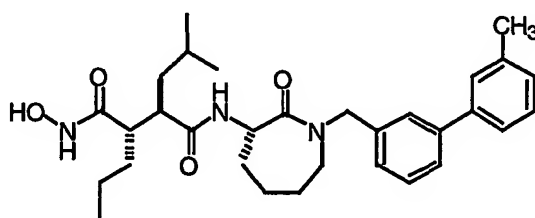
(2R,3S) N1-[(3S)-hexahydro-1-(3-(4-fluorophenyl)benzyl)-2-oxo-1H-azepin-3-yl]-N4-(hydroxy)-2-(2-methylpropyl)-3-(propyl)-butanediamide.



The general procedure reported for Scheme 5 was followed using 4-fluorophenyl boronic acid. Purification afforded 5.0 mg (54%) of the desired product. MS (M+Na)⁺ = 548.

Example 16

(2R,3S) N1-[(3S)-hexahydro-1-(3-(3-methylphenyl)benzyl)-2-oxo-1H-azepin-3-yl]-N4-(hydroxy)-2-(2-methylpropyl)-3-(propyl)-butanediamide.



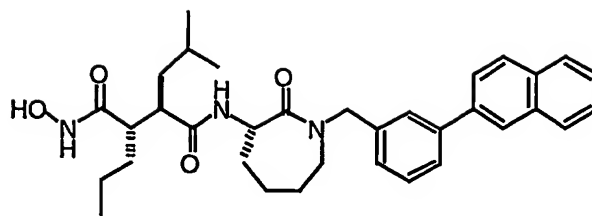
The general procedure reported for Scheme 5 was followed using 3-methylphenyl boronic acid. Purification afforded 3.0 mg (33%) of the desired product. MS (M+Na)⁺ = 544.

25

Example 22

(2R,3S) N1-[(3S)-hexahydro-1-(3-(2-naphthyl)benzyl)-2-oxo-1H-azepin-3-yl]-N4-(hydroxy)-2-(2-methylpropyl)-3-(propyl)-butanediamide.

30

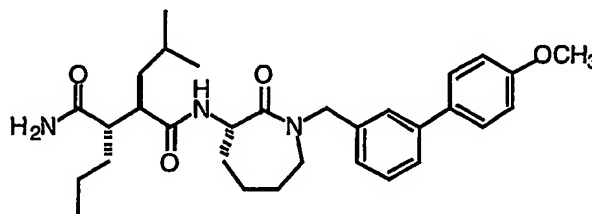


The general procedure reported for Scheme 5 was followed using 2-naphthyl boronic acid. Purification
5 afforded 3.0 mg (31%) of the desired product. MS (M+Na)⁺ = 580.

It will be understood by one skilled in the art that
10 Scheme 6 can be followed in a manner analogous to the procedure for Scheme 5.

Example 23

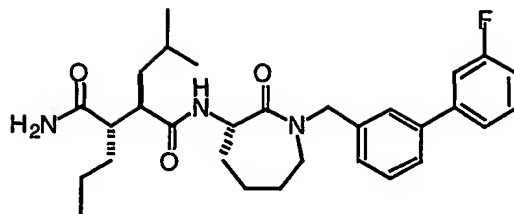
(2R,3S) N1-[(3S)-hexahydro-1-(3-(4-methoxyphenyl)benzyl)-2-
15 oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-
butanediamide.



The general procedure reported for Scheme 6 was
20 followed using 4-methoxyphenyl boronic acid. Purification
afforded 0.5 mg of the desired product. MS (M+Na)⁺ = 544.

Example 24

(2R,3S) N1-[(3S)-hexahydro-1-(3-(3-fluorophenyl)benzyl)-2-
25 oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-
butanediamide.

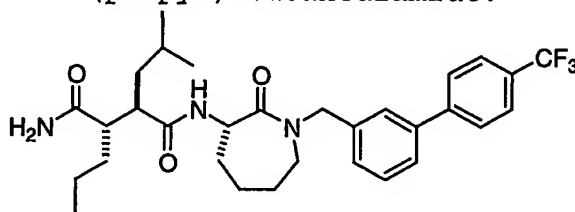


The general procedure reported for Scheme 6 was followed using 3-fluorophenyl boronic acid. Purification afforded 1.6 mg of the desired product. MS (M+Na)⁺ = 532.

5

Example 25

(2R,3S) N1-[(3S)-hexahydro-1-(3-(4-trifluoromethylphenyl)-benzyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.



10

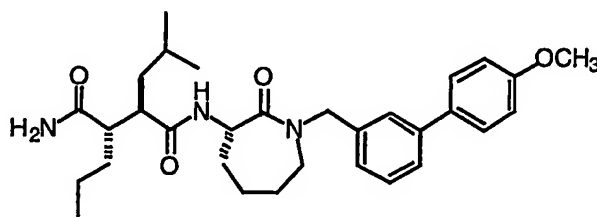
The general procedure reported for Scheme 6 was followed using 4-trifluoromethylphenyl boronic acid. Purification afforded 2.0 mg (40%) of the desired product.

15 MS (M+Na)⁺ = 582.

Example 26

(2R,3S) N1-[(3S)-hexahydro-1-(3-(4-methoxyphenyl)benzyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.

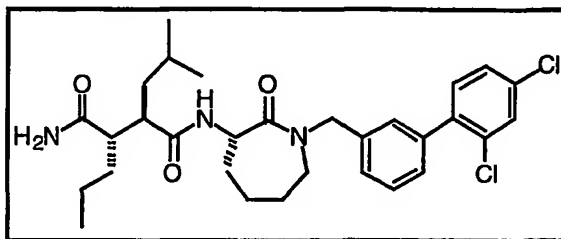
20



The general procedure reported for Scheme 6 was followed using 4-methoxyphenyl boronic acid. Purification
25 afforded 0.5 mg of the desired product. MS (M+Na)⁺ = 544.

Example 27

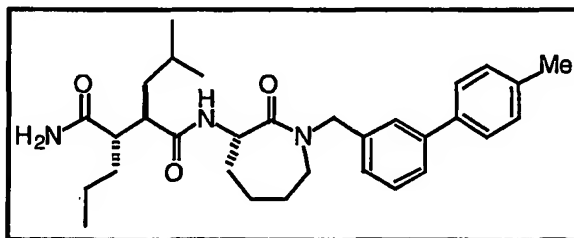
5 (2R,3S) N1-[(3S)-hexahydro-1-(3-(2,4-dichlorophenyl)benzyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.



The general procedure reported for Scheme 6 was followed using 2,6-dichlorophenyl boronic acid. Purification afforded 1.8 mg (11 %) of the desired product. MS (M+Na)⁺ = 582.

Example 28

15 (2R,3S) N1-[(3S)-hexahydro-1-(3-(4-methylphenyl)benzyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.

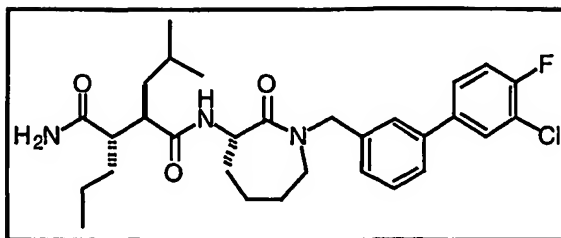


20 The general procedure reported for Scheme 6 was followed using 4-tolyl boronic acid. Purification afforded 1.8 mg (12 %) of the desired product. MS (M+Na)⁺ = 528.

25

Example 29

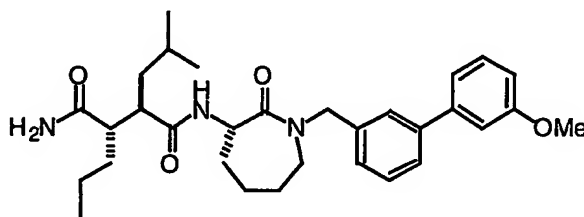
(2R,3S) N1-[(3S)-hexahydro-1-(3-(3-chloro-4-fluorophenyl)benzyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.



The general procedure reported for Scheme 6 was followed using 4-fluoro-3-chlorophenyl boronic acid. Purification afforded 0.5 mg (3.3 %) of the desired product. MS (M+Na)⁺ = 567.

Example 30

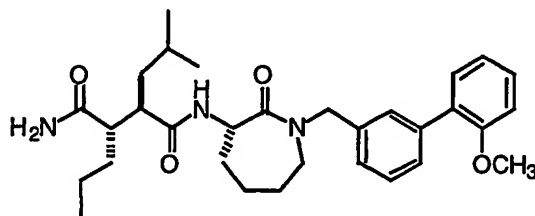
(2R,3S) N1-[(3S)-hexahydro-1-(3-(3-methoxyphenyl)benzyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.



The general procedure reported for Scheme 6 was followed using 2-methoxyphenyl boronic acid. Purification afforded 0.8 mg (5.3 %) of the desired product. MS (M+Na)⁺ = 544.

Example 31

(2R,3S) N1-[(3S)-hexahydro-1-(3-(2-methoxyphenyl)benzyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.



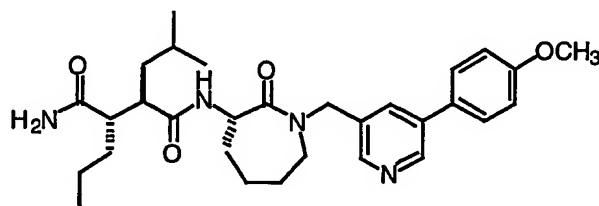
The general procedure reported for Scheme 6 was followed using 2-methoxyphenyl boronic acid. Purification

afforded 1.5 mg (10 %) of the desired product. MS (M+Na)⁺ = 544.

It will be understood by one skilled in the art that
5 Scheme 7 can be followed in a manner analogous to the
procedure for Schemes 5 and 6.

Example 32

(2R,3S) N1-[(3S)-hexahydro-1-(3-(4-methoxyphenyl)pyrid-5-ylmethyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.
10



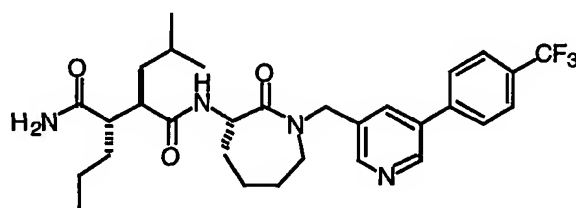
Amide **35** of Scheme 7 (0.10 g, 0.18 mmol) was dissolved
15 in 5 mL of toluene and 41 mg (0.27 mmol) of 4-methoxyphenyl
boronic acid was added, followed by 31 mg (0.0147 mmol) of
tetrakis(triphenylphosphine)palladium, 0.5 mL of a 2M
sodium carbonate solution and 0.5 mL of methanol. The
reaction solution was heated to reflux for 16 h and then
20 allowed to cool to rt. The reaction solution was diluted
with 10 mL of water and extracted 2 x with 50 mL of ethyl
acetate. The combined organic layers were dried and
concentrated and the resulting oil was purified by
chromatography eluting with 30 to 100% ethyl acetate in
25 hexanes as a solvent to provide 30 mg (29%) of biaryl
product. MS (M+H)⁺ = 580.

The purified biaryl product was dissolved in 10 mL of
1:1 trifluoroacetic acid/CH₂Cl₂ and stirred at rt for 2 h.
The solvents were then removed under reduced pressure and
30 the resulting oil was redissolved in 5 mL of toluene and
reconcentrated to remove residual TFA. The crude acid (25
mg, 0.047 mmol) was then dissolved in 1 mL of DMF and 10 µL
of N-methylmorpholine (0.094 mmol) and 42 mg (0.062 mmol)
HATU were added and the reaction solution was stirred at rt

for 45 min. Gaseous ammonia was then bubbled in at a gentle rate for about 1 minute and the solution was stirred for an additional 1 min. The reaction solution was then diluted with 10 mL of water and extracted 3 x with 30 mL of ethyl acetate. The combined organic layers were dried and concentrated under reduced pressure to a solid which was purified by reversed phase HPLC to provide 3.5 mg (10%) of the compound of Example 30 as its trifluoroacetic acid salt. MS (M+H)⁺ = 523.

Example 33

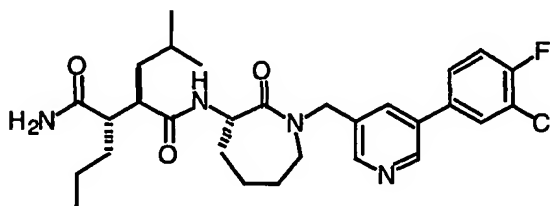
(2R,3S) N1-[(3S)-hexahydro-1-(3-(4-trifluoromethylphenyl)pyrid-5-ylmethyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.



The general procedure reported for the compound of Example 32 was followed using 4-trifluoromethylphenyl boronic acid. Purification by HPLC afforded 6.0 mg of the desired product from as its trifluoroacetic acid salt. MS (M+Na)⁺ = 583.

Example 34

(2R,3S) N1-[(3S)-hexahydro-1-(3-(3-chloro-4-fluorophenyl)pyrid-5-ylmethyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.



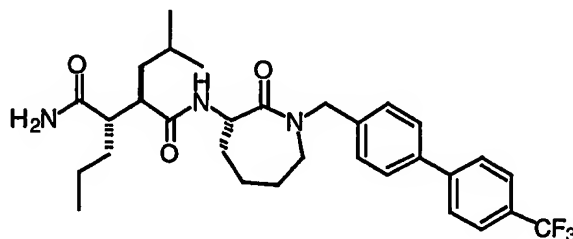
Amide **35** (0.30 g, 0.54 mmol) was dissolved in 3 mL of DMF and 123 mg (0.70 mmol) of 4-methoxyphenyl boronic acid

was added, followed by 44 mg (0.0543 mmol) of bis(diphenylphosphinoferrocene) palladium (II) dichloride and 1.0 mL (7.18 mmol) of triethylamine. The reaction solution was heated to 80°C for 24 h and then allowed to cool to rt. The reaction solution was diluted with 10 mL of water and extracted 2 x with 50 mL of ethyl acetate. The combined organic layers were dried and concentrated and the resulting oil was purified by chromatography eluting with 20 to 100% ethyl acetate in hexanes as a solvent to provide 140 mg (50%) of biaryl product. MS (M+Na)⁺ = 624.

The general procedure reported for the compound of Example 32 was then followed to provide the amide. Purification by chromatography eluting with 20 to 100% ethyl acetate in hexanes afforded 45 mg of the desired product of Example 34 as its trifluoroacetic acid salt. MS (M+Na)⁺ = 567.

Example 39

(2R,3S) N1-[(3S)-hexahydro-1-(4-(4-trifluoromethylphenyl)-benzyl)-2-oxo-1H-azepin-3-yl]-2-(2-methylpropyl)-3-(propyl)-butanediamide.



Step (39-a) 3-(1,1-dimethylethylcarbomethoxy-N-(4-bromophenylmethyl)caprolactam.

The title compound was synthesized in a manner analogous to the preparation of 3-(1,1-dimethylethylcarbomethoxy-N-(benzophenone-3-ylmethyl)caprolactam in Example 10 but using 4-bromobenzyl bromide as the alkylating agent. The compound was purified by chromatography eluting with 5-20% ethyl acetate in

hexanes as eluent to provide 7.0 g (70%) of the title compound as a solid. MS (M+Na)+ = 419.

Step (39-b) 3-(1,1-dimethylethylcarbomethoxy-*N*-(4, -(4'-trifluoromethylphenyl)phenylmethyl)caprolactam.

To a solution of 3-(1,1-dimethylethylcarbomethoxy-*N*-(4-bromophenylmethyl)caprolactam (0.5 g, 1.26 mmol) dissolved in 10 mL of toluene was added 263 mg (1.38 mmol) of 4-trifluoromethylphenyl boronic acid, 1 mL of methanol, and 1 mL of a 2M solution of potassium carbonate. The solution was degassed by nitrogen bubbling for 5 min, and then 33 mg of tris(dibenzylideneacetone)dipalladium(0) chloroform adduct and 66 mg of triphenylphosphine was added. The solution was heated to reflux for 16 h and then allowed to cool and diluted with 20 mL of water. The aqueous layer was extracted 3 x with 25 mL of ethyl acetate and concentrated. The resulting oil was purified by chromatography eluting with 20% ethyl acetate in hexanes to afford 0.47g (81%) of an oil which crystallized on standing.

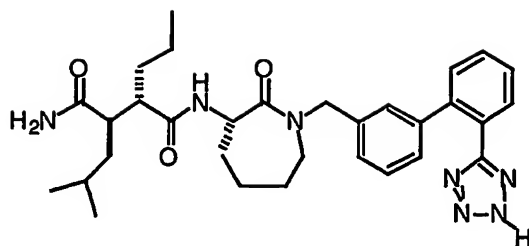
Step (39-d) The title compound, Example 39, was synthesized in a manner analogous to the synthesis of the compound of Example 8 using succinate 10 (280 mg, 1.04 mmol) and 3-(1,1-dimethylethylcarbomethoxy-*N*-(4, -(4'-trifluoromethylphenyl)-phenylmethyl)caprolactam. The compound was purified by chromatography eluting with 20-100% ethyl acetate in hexanes to afford 40 mg of a white powder. MS (M+H)+ = 560.

30

Example 40

(2S,3R) N1-[(3S)-hexahydro-1-(3-(2-tetrazolylphenyl)benzyl)-2-oxo-1H-azepin-3-yl]-2-(propyl)-3-(2-methylpropyl)-butanediamide.

35

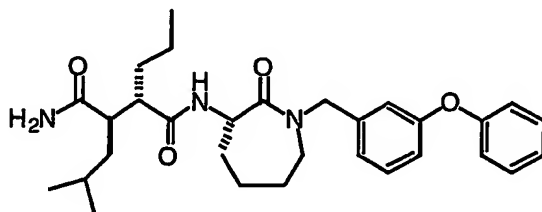


Step (40-a): The compound of Example 40 was synthesized in a manner analogous to the synthesis of the compound of Example 39, but using the substituted acid 28 of Scheme 6 (50 mg, 0.10 mmol) and *o*-((*N*-trityl)-tetrazole)phenylboronic acid under the conditions for the formation of the compound (39-b). The desired biaryl acid was isolated as an impure mixture (134 mg) and used directly in Step (40-b).

Step (40-b): The acid from Step (40-a) (134 mg, impure mixture) was converted to the amide under the conditions reported for the compound of Example 7. The crude amide was then dissolved in 2 mL of 10% trifluoroacetic acid in methanol and allowed to stir at rt for 30 min. The solvents were removed and the residue was purified by chromatography eluting with 10% methanol in ethyl acetate to provide 40 mg (71%, 2 steps) of the compound of Example 40 as a sticky powder. MS (M+Na)+ = 582.

Example 41

(2*S*,3*R*) N1-[(3*S*)-hexahydro-1-(3-phenoxybenzyl)-2-oxo-1*H*-azepin-3-yl]-2-(propyl)-3-(2-methylpropyl)-butanediamide.



Step (41-a): The compound of Example 41 is formed by coupling Succinate 23 (480 mg, 1.21 mmol) with the substituted caprolactam TFA salt 2c under the conditions

reported for the synthesis of the compound of Example 8. The crude fluorenylmethyl ester was used in the next step with out further purification. MS (M+Na)+ = 709.

5 Step (41-b): The crude fluorenylmethyl ester is dissolved in 2 mL of a 50% solution of piperidine in CH₂Cl₂ and stirred for 3 h at rt. A 10 mL portion of 1N HCl was then added and the mixture was extracted 3 x with 10 mL of ethyl acetate. The crude acid was used in the next step with out further purification. MS (M+H)+ = 509.

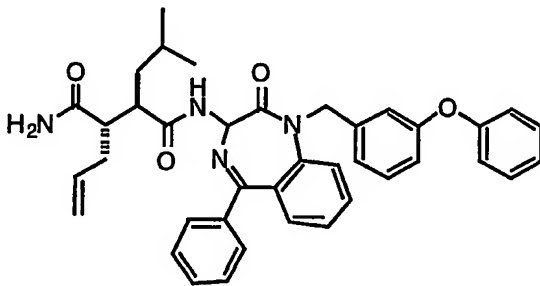
10 The compound of Example 41 was then prepared using the acid from Step (41-b) under the conditions reported for compound of Example 7. The compound was purified by chromatography eluting with 5% methanol in CH₂Cl₂ to afford 120 mg (19%, 3 steps) of a white powder. MS (M+H)+ = 508.

15

Example 42

(2S,3R) N1-[1,3-dihydro-1-(3-phenoxybenzyl)-2-oxo-5-(phenyl)-2H-1,4-benzodiazepin-3-yl]-2-(2-methylpropyl)-3-(allyl)-butanedi-
20 amide.

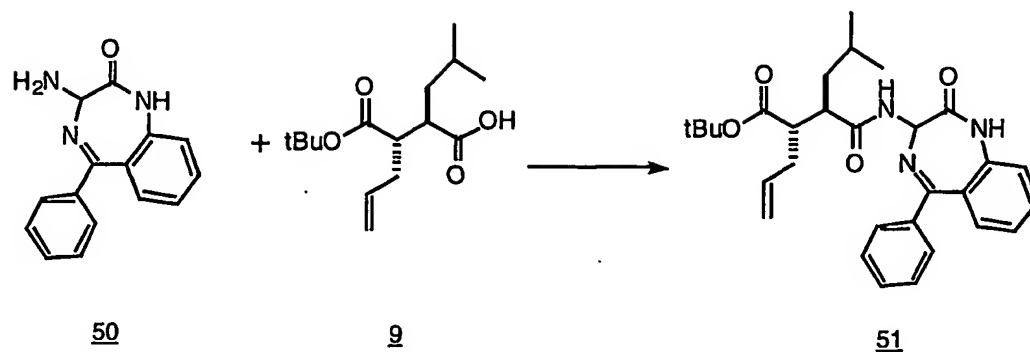
20



Step (42-a) 3-Phenoxybenzyl iodide:

To a solution of 3-phenoxybenzyl chloride (10.0 g, 45.7mmol) in 200 ml acetone was added sodium iodide (7.6 g, 507mmol). The mixture was stirred at temperature overnight. The mixture was diluted with 300ml hexane and the organic layer was washed twice with 5% sodium bicarbonate, once with brine and then dried over MgSO₄.
30 Evaporation of the filtrate gave a light yellow oil. The product was used in next step without purification. ¹H NMR (CDCl₃) 4.4 (s, 2H), 6.8-7.4 (m, 9H).

Step (42-b):



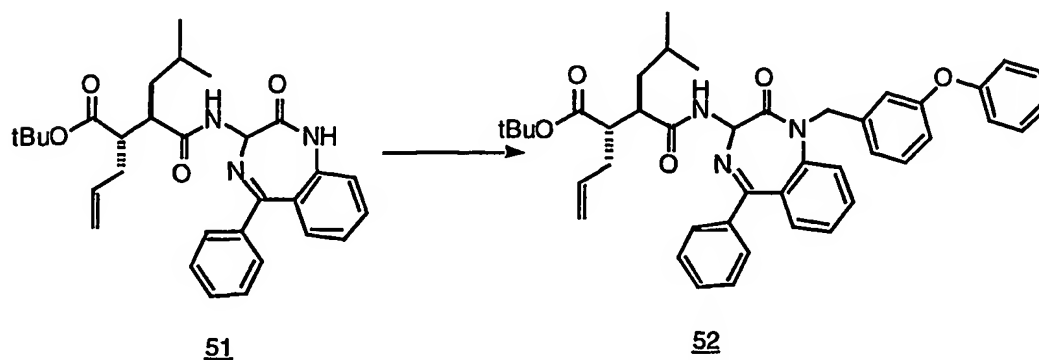
5

To a solution of benzodiazepine 50 (910 mg, 3.63mmol), succinate 9 (980 mg, 3.63mmol), hydroxybenzotriazole (980 mg., 7.25mmol) and EDC (870 mg, 4.54mmol) in 100 ml CH₂Cl₂ at 0 degrees was added triethylamine (0.76 ml, 5.45mmol).

10 The reaction mixture was washed with saturated sodium bicarbonate solution, 1.0N HCl, brine and dried over MgSO₄. Evaporation of the organic layer and purification by column chromatography on silica gel with hexane-ethyl acetate (7:3) gave 610 mg of benzodiazepine 51 as a white solid.

15 M+H = 504.37. ¹H NMR (CDCl₃) 0.8-1.0 (m, 6H), 1.0-1.2 (m, 1H), 1.4-1.5 (d, 9H), 1.6-1.9 (m, 2H), 2.2-2.8 (m, 4H), 4.9-5.2 (m, 2H), 5.6 (dd, 1H), 5.6-6.0 (m, 1H), 7.0-7.6 (m, 9H).

20 Step (42-c):



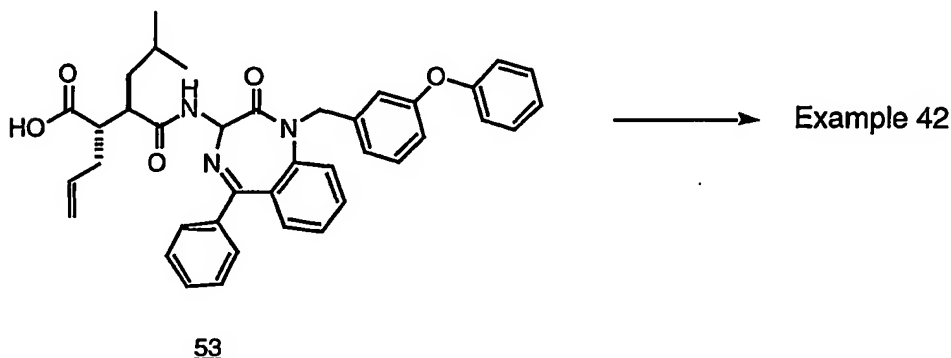
To a solution of benzodiazepine 51 (440 mg, 0.875mmol) in DMF (20 ml) at 0 degrees was added NaH (45 mg, 1.12mmol). The mixture was stirred at 0 degrees for 1.5 hr and then a solution of 3-phenoxybenzyl iodide (330 mg, 1.06mmol) in 10 ml DMF was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred overnight. TLC using hexanes:EtOAc 6:4 (product Rf = 0.31) indicated that the reaction was complete. The reaction mixture was quenched with water, and the solvent was evaporated under high vacuum, which provided a viscous yellow oil. The product benzodiazepine 52 was dissolved in ethyl acetate, which was washed with water (2x), brine and then dried over MgSO₄.

Evaporation of solvent gave 600mg of benzodiazepine 52 as a yellow oil which was not further purified. M+H = 686.3, M+Na = 708.3. ¹H NMR (CDCl₃) 0.8-1.0 (m, 6H), 1.0-1.3 (m, 1H), 1.4-1.5 (d, 9H), 1.5-1.9 (2H), 2.2-2.7 (4H), 4.6-4.8 (d, 1H), 4.9-5.2 (m, 2H), 5.6-5.9 (m, 3H), 6.6-7.6 (m, 18H).

A solution of benzodiazepine 52 in 40 ml of TFA/CH₂Cl₂ (1:1) was stirred overnight at room temperature then evaporated to dryness. Repeated addition of toluene and evaporation provided 560 mg. of 53 as a yellow solid. (M - H = 629.1)

25

Step (42-d):



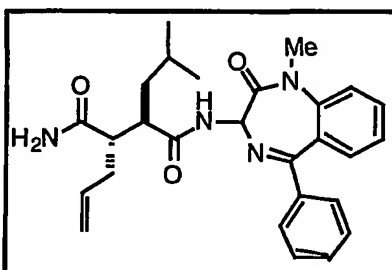
To a solution of benzodiazepine 53 and HATU (410mg, 1.08mmol) in 30 ml DMF was added diisopropylethylamine (0.6

ml, 3.44mmol) at 0 degrees. After 10 minutes, ammonia gas was bubbled through the solution for two minutes, and the reaction mixture was allowed to warm to room temperature and stirred overnight. Addition of water and solvent
 5 evaporation under high vacuum provided a yellow solid. The solid was taken up in ethyl acetate-water (1:1), and the organic layer was washed with water (2x), brine and then dried over MgSO₄. Evaporation of solvent gave a light
 10 yellow solid. Chromatographic purification on silica gel using CH₂Cl₂ : methanol (10:0.5) gave 256 mg of Example 42. M+H = 629.2 HNMR (CDCl₃) 0.8-1.0 (m, 6H), 1.2-1.4 (m, 1H), 1.6-2.0 (m, 2H), 2.2-2.8 (4H), 4.6-4.8 (m, 1H), 5.0-5.2 (m, 2H), 5.6-5.9 (m, 3H), 6.2-7.8 (m, 18H).

15

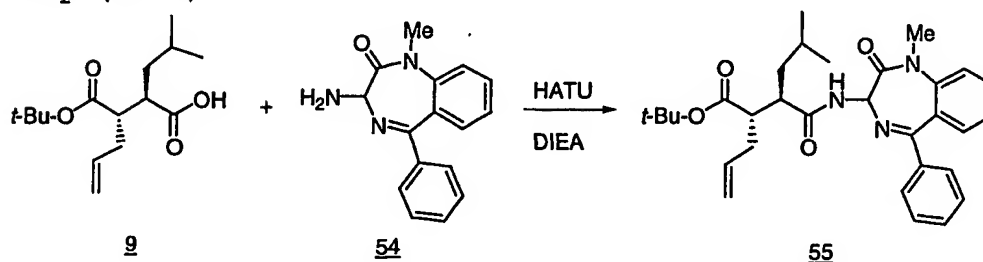
Example 43

(2S,3R) N1-[1,3-dihydro-1-methyl-2-oxo-5-(phenyl)-2H-1,4-benzodiazepin-3-yl]-2-(2-methylpropyl)-3-(allyl)-butanediamide.



20

Step (43-a):



25

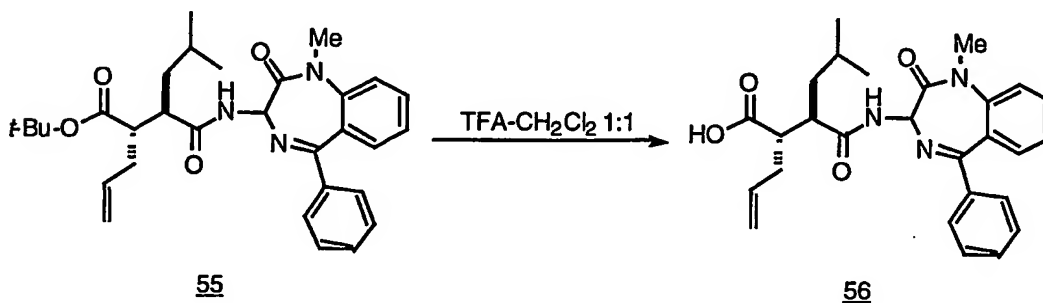
A solution of tert-butyl succinate ester 9 (1.1 eq.) in DMF (0.25 M) under N₂ at 0°C was added HATU (1.1 eq.), then Hunig's base (4.0eq.). The mixture was stirred at 0°C

for 10 mins. A solution of 2,3-dihydro-1-methyl-3-amino-5-phenyl-1H-1,4-benzodiazepin-2-one 54 in DMF (0.8 M) (1.0 eq.) was added to this solution. The reaction mixture was stirred overnight at room temperature and then transferred to a separatory funnel containing water. 30% n-Hexane in ethyl acetate was added which gave a clear organic layer. The aqueous solution was extracted twice with 30% n-hexane in ethyl acetate. The combined organic layers were washed with water and brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was purified by chromatography on flash grade silica gel using 20% ethyl acetate in n-hexane. The compound 55 was isolated as an amorphous white solid (85%). Rf = 0.25 (7:3 n-hexane:ethyl acetate).

¹H-NMR:(CDCl₃): δ 7.61-7.21 (m, 10H); 5.77-5.73 (m, 1H); 5.57-5.54 (d, 1H); 5.20-4.97 (m, 2H); 3.47 (s, 3H); 2.63-2.33 (m, 4H); 1.80-1.76 (m, 2H); 1.47-1.46 (d, 9H); 1.43-1.11 (m, 1H); 1.01-0.86 (m, 6H).

MS: C₃₁H₃₉N₃O₄ (M+H) 518.3 (M+Na) 540.3.

Step (43-b):



A solution of 55 in 50% TFA in methylene chloride (0.15M) was stirred at room temperature overnight. The solution was concentrated *in vacuo*, washed and concentrated four times with toluene *in vacuo* to give compound 56 as an amorphous solid (95%). Rf = 0.64 (9.5 : 0.5 methylene chloride : methanol). MS: C₂₇H₃₁N₃O₄ (M+H) 462.

Step (43-c):



5 To a solution of **56** (1.0 eq.) in DMF (0.25 M) under N₂ at 0°C was added HATU (1.1 eq.), and then Hunig's base (4.0eq.). The mixture was stirred at 0°C for 10 mins, and then anhydrous ammonia bubbled through the solution for two minutes. The reaction mixture was stirred overnight at

10 room temperature and then transferred to a separatory funnel containing water and diluted with 30% n-hexane in ethyl. The aqueous solution was extracted twice with 30% n-hexane in ethyl acetate. The combined organic layers were washed with water and brine, dried over magnesium sulfate, and

15 concentrated *in vacuo*. The residue was purified by chromatography on flash grade silica gel using 4% methanol in methylene chloride. The title compound, Example 43, was isolated as an amorphous white solid (87%). R_f = 0.43 (9:1 methylene chloride:methanol).

20

¹H NMR:(CDCl₃): δ 7.63-7.22 (m, 10H); 6.25-6.13 (d, 1H); 5.88-5.73 (m, 1H); 5.53-5.51 (dd, 1H); 5.44-5.41 (d, 1H); 5.22-5.04 (m, 2H); 3.47-3.46 (d, 3H); 2.74-2.31 (m, 4H); 1.81-1.61 (m, 2H); 1.34-1.22 (m, 1H); 0.99-0.87 (m, 6H).

25

MS: C₂₇H₃₂N₄O₃ (M+H) 461.

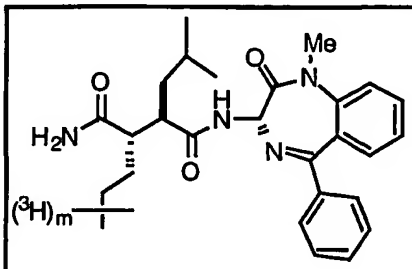
It is understood that the (R) or (S)-benzodiazepine diastereomer of Example 43 can be prepared using methods

30 analogous to the present example but employing the (R) or (S) stereoisomer of intermediate **2a** in Step (43-a), respectively.

Example 43T

Tritiated (2S,3R) N1-[1,3-dihydro-1-methyl-2-oxo-5-(phenyl)-2H-1,4-benzodiazepin-3-yl]-2-(2-methylpropyl)-3-(n-propyl)-butanediamide.

5



Example 43T was synthesized by reducing the double bond present in the (S)-benzodiazepine diastereomer of Example 43. The (S) diastereomer of Example 43 may be separated from the product of Step (43-c) by means known to one skilled in the art and the single isomer reduced. Alternatively, this diastereomer may be prepared directly as stated above. Thus, the (S)-benzodiazepine diastereomer of Example 43 was dissolved in tetrahydrofuran and hydrogenated using tritium gas, by methods known to one skilled in the art organic synthesis. Purification by reverse phase HPLC on a Vydac-18 column provided the desired tritiated amide Example 43T wherein m is approximately 2.

It is understood that one skilled in the art of organic synthesis can synthesize radiolabeled compounds of the present invention for use as a tagged inhibitor of beta-amyloid production using radiolabeling techniques well know in the art. For example tritiation, using catalysts such as Pd/C or Wilkinson's catalyst and $^3\text{H}_2$ gas, one skilled in the art can reduce olefin precursors. Examples of olefin precursors are Examples 8, 10, 42, 43, intermediate Succinate 10 and intermediate Benzodiazepine 51.

Representative Procedures for the Synthesis of Radiolabeled
Compounds

Described below are representative procedures for the
5 synthesis of radiolabeled compounds. These nonlimiting
representative procedures, and other procedures known in
the art, will be readily known and appreciated by one of
skill in the art of organic synthesis of radiolabeled
compounds. The radiolabeled ligands of macromolecules
10 involved in the process of APP and/or beta-amyloid
production (the "radiolabeled ligands") of the present
invention can be synthesized using standard synthetic
methods known to those skilled in the art, using
radioisotopes of halogens (such as chlorine, fluorine,
15 bromine and iodine), as well as others. Radioisotopes
include ^{123}I , ^{125}I , ^{131}I , $^{99\text{m}}\text{Tc}$, and ^{111}In .

The radiolabeled ligands of the invention may be
labeled either directly (that is, by incorporating the
radiolabel directly into the compounds) or indirectly (that
20 is, by incorporating the radiolabel into the compounds
through a chelator which has been incorporated into the
compounds. For brain imaging, it is expected that direct
labeling will be preferred in the present invention. For
direct labeling, as those skilled in the art will
25 recognize, the labeling may be isotopic or nonisotopic.
With isotopic labeling, one group already present in the
compound is substituted with (exchanged for) the
radioisotope. With nonisotopic labeling, the radioisotope
is added to the compound without substituting with
30 (exchanging for) an already existing group.

Generally, labeled compounds are prepared by
procedures which introduce the labeled atom at a late stage
of the synthesis. This allows for maximum radiochemical
yields, and reduces the handling time of radioactive
35 materials. When dealing with short half-life isotopes, a
major consideration is the time required to conduct
synthetic procedures, and purification methods. Protocols
for the synthesis of radiopharmaceuticals are described in

Tubis and Wolf, Eds., "Radiopharmacy", Wiley- Interscience, New York (1976); Wolf, Christman, Fowler, Lambrecht, "Synthesis of Radiopharmaceuticals and Labeled Compounds Using Short-Lived Isotopes", in Radiopharmaceuticals and Labeled Compounds, Vol 1, p. 345-381 (1973), the disclosures of each of which are hereby incorporated herein by reference, in their entirety.

Various procedures may be employed in preparing the radiolabeled compounds of the invention where the radiolabel is a halogen. Some common synthetic methodologies for isotopic halogen labeling of aromatic compounds such as the type present here are iododediazonization, iododeborobation, iododestannylation, iododesilation, iododethallation, and halogen exchange reactions. The most common synthetic methodology for nonisotopic halogen labeling of aromatic compounds such as the type present here is iododeprotonation or electrophilic aromatic substitution reactions. These methods and additional procedures are described in Merkushev, Synthesis, 923 (1988), and Seevers et al., Chem. Rev., 82: 575 (1982), the disclosures of each of which are hereby incorporated herein by reference, in their entirety.

By way of example, isotopically radiolabeled 4, 5 and 6-halo t-butyloxycarbonyl-3-aminomethylbenzoic acid derivatives may be prepared using the general procedures described above for the synthesis of the unlabeled compounds. In carrying out such radiolabeling, it is important that the half-life of the isotope chosen be much longer than the handling time of the reaction sequences. Known starting materials include the 2, 3, and 4-iodo (^{123}I , ^{125}I , and ^{131}I) benzoic acids. Iodo-radiolabeled compounds may also be isotopically prepared from anilines by the Sandmeyer reaction as described in Ellis et al., Aust. J. Chem., 26: 907 (1973).

Alternatively, radiolabeled compounds may prepared by way of isotopic labeling from an unlabeled bromo or iodo derivatives by various two step reaction sequences, such as through the use of trialkylsilyl synthons as described in

Wilson et al., J. Org. Chem., 51: 483 (1986) and Wilbur et al., J. Label. Compound. Radiopharm., 19: 1171 (1982), the use of trialkylsilyl synthons as described in Chumpradit et al. J. Med. Chem., 34: 877 (1991) and Chumpradit et al. J. Med. Chem., 32: 1431 (1989), and the use of boronic acid synthons as described in Kabalka et al., J. Label. Compound. Radiopharm., 19: 795 (1982) and Koch et al., Chem. Ber., 124:2091 (1991).

10 In preparing radiolabeled compounds of the present invention, to maximize radiochemical yields, to reduce the handling time of radioactive materials, and to prepare short half-life halogen labeled compounds, it is preferable to perform the isotopic halogen labeling as one of the
15 final steps in the compound synthesis. The following provides exemplary procedures for such late stage labeling.

Unlabeled iodo compounds are versatile precursors which can be converted to the labeled derivatives by any of the two step reaction sequences described above. In
20 general, useful functionalities to incorporate into a compound includes bromo, the nitro, the trialkylsilyl, the trialkyltin, and the boronic acid groups. The synthesis and application of each of these precursors is described above. Radioiodination of a compound of the present
25 invention may be achieved via isotopic labeling during the final stages of preparation by the substitution of radioactive iodide for a stable iodine atom already present in the molecule. This can often be done by heating the compound with radioactive iodide in an appropriate solvent
30 as described in Ellis et al., Aust. J. Chem., 26: 907 (1973).

In some cases radiolabeled compounds of the present invention may also be isotopically iodo-labeled during the final stages of their preparation from anilines by the
35 Sandmeyer reaction as described in Ellis et al., Aust. J. Chem., 26: 907 (1973). Alternatively, a compound may be isotopically labeled late in the reaction scheme from the unlabeled bromo or iodo derivatives by various two step

reaction sequences, as described above, such as through the use of trialkylsilyl synthons as described in Wilson et al., J. Org. Chem., 51: 4833 (1986) and Wilbur et al., J. Label. Compound. Radiopharm., 19: 1171 (1982), through the
5 use of trialkylsilyl synthons as described in Chumpradit et al., J. Med. Chem., 34: 877 (1991) and Chumpradit et al., J. Med. Chem., 32: 1431 (1989), and through the use of boronic acid synthons as described in Kabalka et al., J. Label. Compound. Radiopharm., 19: 795 (1982) and Koch et
10 al., Chem. Ber., 124:2091 (1991).

A related approach where the isotopic halogen radiolabeling may be carried out late in the synthesis scheme involves converting a synthetic intermediate compound that already incorporates a trialkylsilyl,
15 trialkyltin, or boronic acid groups. Labeled iodo derivatives may also be readily prepared nonisotopically from the amino, hydroxy, or methoxy substituted compounds as described in Arora et al J. Med. Chem., 30:918 (1987). Electrophilic aromatic substitution
20 reactions are enhanced by the presence of such electron-donating substituents.

Another representative approach to the incorporation of a radiolabeled halogen in compounds containing methyl substituted phenyl involves the conversion to a a-
25 halotoluene derivative with NBS or NCS under free-radical halogenation conditions. The benzylic halides may be smoothly replaced by radiolabeled iodide through a nucleophilic substitution reaction. The above described process chemistry can also be used to prepare any
30 radioactive halogen isotope.

By way of illustration, ^{18}F derivatives of certain compounds can be prepared by conjugation of ^{18}F functionalized phenyl intermediates (R.H. Mach et al., J. Med. Chem., 1993, 36,3707-3720).
35

UTILITY

A β production has been implicated in the pathology of Alzheimer's Disease (AD). The compounds of the present

invention as well as compounds determined from the present invention have utility for the prevention and treatment of AD by inhibiting the proteolytic activity leading to A β production. Methods of treatment target formation of A β production through the enzymes involved in the proteolytic processing of β amyloid precursor protein. Compounds that inhibit β or γ secretase activity, either directly or indirectly, control the production of A β . Such inhibition of β or γ secretases reduces production of A β , and is expected to reduce or prevent the neurological disorders associated with A β peptide, such as Alzheimer's Disease.

Cellular screening methods for inhibitors of A β production, testing methods for the *in vivo* suppression of A β production, and assays for the detection of secretase activity are known in the art and have been disclosed in numerous publications, including PCT publication number WO 98/22493, EPO publication number 0652009, US patent 5703129 and US patent 5593846; all hereby incorporated by reference.

The compounds of the present invention as well as compounds determined from the present invention have utility for the prevention and treatment of disorders involving A β production, such as cerebrovascular disorders.

Compounds of Formula (I) are expected to possess γ -secretase inhibitory activity. The γ -secretase inhibitory activity of the compounds of the present invention is demonstrated using assays for such activity, for example, using the assay described below. Compounds within the scope of the present invention have been shown to inhibit the activity of γ -secretase, as determined using assays for such activity.

Compounds provided by this invention should also be useful as standards and reagents in determining the ability of a potential pharmaceutical to inhibit A β production. These would be provided in commercial kits comprising a compound of this invention.

As used herein " μ g" or "ug" denotes microgram, "mg" denotes milligram, "g" denotes gram, " μ L" denotes

microliter, "mL" denotes milliliter, "L" denotes liter, "nM" denotes nanomolar, "μM" or "uM" denotes micromolar, "mM" denotes millimolar, "M" denotes molar, "nm" denotes nanometer, "SDS" denotes sodium dodecyl sulfate, and "DMSO" denotes dimethyl sulfoxide, and "EDTA" denotes ethylenediaminetetraacetate.

A compound is considered to be active if it has an IC₅₀ or K_i value of less than about 100 μM for the inhibition of Aβ production or inhibition of proteolytic activity leading to Aβ production. Compounds, as demonstrated by use of the invention, have demonstrated IC₅₀ values, for the inhibition of Aβ production, of less than about 100 μM. Preferably compounds, as demonstrated by use of the invention, demonstrate IC₅₀ values, for the inhibition of Aβ production, of less than about 1 μM. More preferably compounds, as demonstrated by use of the invention, demonstrate IC₅₀ values, for the inhibition of Aβ production, of less than about 100 nM. Even more preferably compounds, as demonstrated by use of the invention, demonstrate IC₅₀ values, for the inhibition of Aβ production, of less than about 50 nM.

β Amyloid Precursor Protein Accumulation Assay (βAPPA assay)

An assay to evaluate the accumulation of Aβ protein was developed to detect potential inhibitors of secretases. The assay uses the CHO N 9 cell line, characterized for expression of exogenous APP by immunoblotting and immunoprecipitation.

The effect of test compounds on the accumulation of Aβ in the conditioned medium is tested by immunoprecipitation. N 9 cells are grown to confluency in 6-well plates and washed twice with 1 x Hank's buffered salt solution. The cells are starved in methionine/cysteine deficient media for 30 min., followed by replacement with fresh deficient media containing 150uCi Tran35S-LABEL™ (ICN). Test compounds dissolved in DMSO (final concentration 1%) are added, over a range of 1 picomolar to 100 micromolar,

together with the addition of the fresh media containing Tran35S-LABEL™. The cells are incubated for 4 h at 37°C in a tissue culture incubator.

At the end of the incubation period, the conditioned
5 medium is harvested and pre-cleared by the addition of 5 µl
normal mouse serum and 50ul of protein A Sepharose
(Pharmacia), mixed by end-over-end rotation for 30 minutes
at 4°C, followed by a brief centrifugation in a microfuge.
The supernatant is then harvested and transferred to fresh
10 tubes containing 5ug of a monoclonal antibody (examples of
antibodies include but are not limited by, clone 1101.1,
directed against an internal peptide sequence in Aβ; or
6E10 from Senetek; or 4G8 from Senetek; additionally
polyclonals from rabbit antihuman Aβ from Boehringer
15 Mannheim) and 50 µl protein A Sepharose. After incubation
overnight at 4°C, the samples are washed three times with
high salt washing buffer (50mM Tris, pH 7.5, 500mM NaCl,
5mM EDTA, 0.5% Nonidet P-40), three times with low salt
wash buffer (50mM Tris, pH 7.5, 150mM NaCl, 5mM EDTA, 0.5%
20 Nonidet P-40), and three times with 10mM Tris, pH 7.5. The
pellet after the last wash is resuspended in SDS sample
buffer (Laemmli U.K. Cleavage of structural proteins during
the assembly of the head of bacteriophage T4. Nature 227,
680-5, 1970.) and boiled for 3 minutes. The supernatant is
25 then fractionated on either 10-20% Tris/Tricine SDS gels or
on 16.5% Tris/Tricine SDS gels. The gels are dried and
exposed to X-ray film or analyzed by phosphorimaging. The
resulting image is analyzed for the presence of Aβ
polypeptides. The steady-state level of Aβ in the presence
30 of a test compound is compared to wells treated with DMSO
(1%) alone. A typical test compound in this assay blocks
Aβ accumulation in the conditioned medium, and is
considered active with an IC₅₀ less than 100 µM.

35 C-Terminus β Amyloid Precursor Protein Accumulation Assay
(CTF assay)

The effect of test compounds on the accumulation of C-terminal fragments is determined by immunoprecipitation of

APP and fragments thereof from cell lysates. N 9 cells are metabolically labeled, as above, with media containing Tran35S-LABEL™, in the presence or absence of test compounds. At the end of the incubation period, the conditioned medium are harvested and cells lysed in RIPA buffer (10 mM Tris, pH 8.0 containing 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 150mM NaCl, 0.125% NaN₃). Again, lysates are precleared with 5ul normal rabbit serum/50ul protein A Sepharose, followed by the addition of BC-1 antiserum (15ul;) and 50ul protein A Sepharose for 16 hours at 4°C. The immunoprecipitates are washed as above, bound proteins eluted by boiling in SDS sample buffer and fractionated by Tris/Tricine SDS-PAGE. After exposure to X-ray film or phosphorimager, the resulting images are analyzed for the presence of C-terminal APP fragments. The steady-state level of C-terminal APP fragments is compared to wells treated with DMSO (1%) alone. A typical test compound in this assay stimulates C-terminal fragment accumulation in the cell lysates, and is considered active with an IC₅₀ less than 100 µM.

Accumulation-Release Assay

This immunoprecipitation assay is specific for γ secretase activity (i.e., proteolytic activity required to generate the C-terminal end of A β either by direct cleavage or generating a C-terminal extended species which is subsequently further proteolyzed). N 9 cells are pulse labeled with media containing Tran35S-LABEL™ in the presence of a reported γ secretase inhibitor (MDL 28170; Higaki J, Quon D, Zhong Z, Cordell B. Inhibition of beta-amyloid formation identifies proteolytic precursors and subcellular site of catabolism. Neuron 14, 651-659, 1995) for 1 h, followed by washing to remove ³⁵S radiolabel and MDL 28170. The media is replaced and test compounds are added over a dose range (for example 0.1nM to 100uM). The cells are chased for increasing periods of times and A β is isolated from the conditioned medium and C-terminal fragments from cell lysates (see accumulation assay above).

The activity of test compounds are characterized by whether a stabilization of C-terminal fragments is observed and whether A β is generated from these accumulated precursor. A typical test compound in this assay prevents the
5 generation of A β out of accumulated C-terminal fragments and is considered active with an IC₅₀ less than 100 μ M.

Radioligand Competition Binding Assay (RCB Assay):

The following assay, of the invention, discloses a
10 novel assay to rapidly screen and evaluate potential inhibitors of secretases. The assay enables screening for inhibitors of A β production or inhibitors of proteolytic activity leading to the production of A β by using a competitive binding assay wherein more than one chemical
15 entity competes for a binding site identified for A β production. For example, in a competitive binding assay of the invention competition occurs between potential A β production inhibitors (i.e. compounds being investigated for inhibitory activity) and a standard known for A β
20 production inhibitory activity which standard has been tagged by a radiolabel. Example 7T radiolabeled with tritium is a standard identified for A β production inhibitory activity; however, any radiolabelled or tagged compound binding to the same site as Example 7T could be
25 used in this assay. It is understood that the theory of competitive binding is well known to one skilled in the art of pharmacology. The compounds identified by this invention may have utility for the prevention and treatment of neurological disorders relating to A β production,
30 including Alzheimer's disease, by inhibiting A β production.

Materials:

Assay buffer: Hepes 50 mM pH 7.0.

Competing compounds/potential inhibitors: weigh and
35 dilute in 100% DMSO at a concentration of 1×10^{-2} M.
From that stock, a second (6×10^{-4} M) stock is made in 100% DMSO. The working stock (6×10^{-5} M) is made from the second in assay buffer containing 6% DMSO.

Wash buffer: Phosphate buffered saline containing 0.01% triton X-100, pH 7.0 at 4°C.

Membrane: HEK₂₉₃ control membranes (Receptor Biology, Inc.), or rat whole brain homogenates prepared as follows: Frozen pellets of approximately 10 mg protein HEK₂₉₃ cell membranes are thawed on ice and homogenized in 10 ml of assay buffer, using a Brinkman Polytron (PT-10) setting 6 for 10 sec. The homogenate was centrifuged at 48,000 X g for 12 minutes and the resulting pellet washed by repeating the homogenization and centrifugation steps. The final cell pellet was resuspended in buffer to yield a protein concentration of approximately 0.35 mg/ml as assayed by the method of Bradford (1976) using bovine serum albumin as the standard.

Rats: for the rat whole brain homogenates, (male Sprague-Dawley rats 200 to 300 g., Charles River) are decapitated and brains dissected on an ice-chilled glass plate. Brains weighing ~2g are homogenized in 20 ml of assay buffer and prepared by the method described above for the cell homogenates. The final pellet is resuspended to yield a protein conc. of ~5 mg/ml original wet weight.

Radiolabeled standard: [³H] **I-7T** (Example 7T; synthesized by Dupont Pharm. Co.) S.A. 87.5 Ci/mMol, (11.43 μM **I-7T**).

Radioligand Competition Binding Assay Method:

Assays are initiated by addition of 150 μl membrane suspension (~0.35 mg protein/ml) to 150 μl of assay buffer containing 1% DMSO, 5 to 30 nM [³H] **I-7T**, and various concentrations of inhibitors over a range of 1 picomolar to 100 micromolar. Binding assays are preformed in duplicate in disposable polypropylene 96 well plates, (Costar Corp., Cambridge, MA) in a final volume of 0.3 ml. Nonspecific binding is defined in the presence of 3 μM **I-7T**. Optimum incubation time at 23°C is 1 hour. The separation of bound radioligand **I-7T** from free radioligand **I-7T** is accomplished

by rapid vacuum filtration of the incubation mixture over GFF glass fiber filters (Inotech Biosystems International, Lansing, MI) presoaked for 2 hours in 0.3% polyethylinamine (pH 13) using an Inotech cell harvester. Filters were
5 washed 2 times with 0.3 ml of ice-cold phosphate buffered saline pH 7.0 containing 0.01% Triton X100. Filters are accessed for radioactivity by liquid scintillation counting using a Packard 2500 TR (Packard Instrument Co., Downers Grove, IL), having a counting efficiency for tritium of
10 ~56%.

Alternatively, it is well known in the art that a homogenous assay format, such as a scintillation proximity assay (SPA), could be employed in the radioligand competition binding assay of the invention. For example,
15 membranes or membrane extracts can be immobilized onto the SPA support, afterwhich the support is then incubated with a tagged inhibitor of beta amyloid production in the presence of a potential inhibitor of beta amyloid production. The SPA support, by nature of its
20 construction, magnifies the radioactive scintillation signal of bound radioactive compounds while not magnifying the radioactive signal of radioactive compounds free in solution. Therefore, the bound tagged inhibitor of beta amyloid production is detected and quantified by
25 scintillation counting in the presence of free tagged inhibitor of beta amyloid production.

It is understood that the process of separating bound tagged inhibitor of beta amyloid production from free tagged inhibitor of beta amyloid production, for example
30 bound radioligand I-7T from free radioligand I-7T, can be conducted in a number of methods. For example the process of separating includes, but is not limited to, filtration or centrifugation. The process of separating is intended to facilitate quantification of bound tagged inhibitor of
35 beta amyloid production. Therefore, the process of separating is also intended to encompass homogeneous techniques, for example SPA, where free tagged inhibitor of beta amyloid production in situ is separated from the

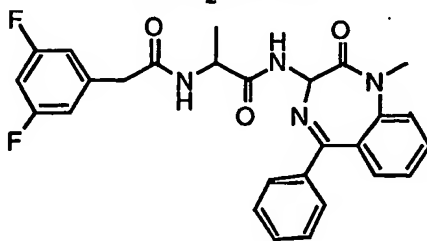
tagged inhibitor of beta amyloid production bound to the solid support of the scintillant. Thus, in a homogeneous technique such as SPA, the free and bound inhibitors are considered separated from each other within the meaning of the invention.

Radioligand Competition Binding Data Analysis:

Resulting disintegrations per minute (dpm's) are expressed as percent inhibition of [³H] I-7T specific binding. IC₅₀ values of competing compounds are calculated using the program GraphPad Prism by GraphPad Software, (San Diego, CA). It is understood that one skilled in the art can determine these values using this program.

A good correlation for inhibition of proteolytic activity leading to A β production has been found between compounds identified in functional assays for determination of A β production, for example the β Amyloid Precursor Protein Accumulation Assay, and compounds identified in the Radioligand Competitive Binding Assay. The correlation is demonstrated by plotting the IC₅₀ values of compounds identified in the functional assay verses the IC₅₀ values of compounds identified in the RCB Assay. Compounds from several chemical series, including Examples disclosed herein, have exhibited, over a range of potencies, similar IC₅₀ values in the RCB Assay as seen in an accumulation assay.

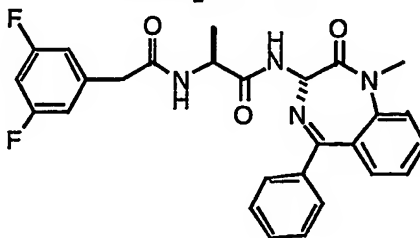
Example 98



30

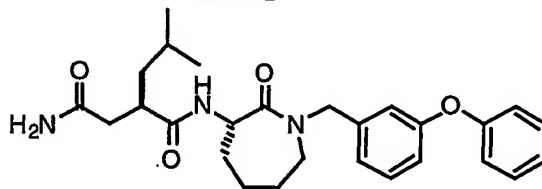
The compound of Example 98 was synthesized according to procedures disclosed in PCT Application WO98/28268, published July 2, 1998.

5

Example 98b

The compound of Example 98b was synthesized according to procedures disclosed in PCT Application WO98/28268, published July 2, 1998.

10

Example 99

15 Step (99a): The compound of Step (99a) is formed by coupling succinate **7** (115 mg, 0.5 mmol) with the substituted caprolactam TFA salt (212 mg, 0.5 mmol) from Step (2c) of Example 2 under the conditions reported for the synthesis of the compound of Example 8. The crude
20 tert-butyl ester was taken on without further purification.

Step (99b): The compound of Step (99b) is formed by dissolving the crude product from Step (99a) in 5 mL of a 1:1 solution of TFA/CH₂Cl₂ and stirring at room temperature for 2 hours. Concentration followed by reconcentration
25 twice from 10 mL of toluene provides the crude acid which was taken on with no further purification.

Step (99c): The title compound, Example 99, was prepared using the acid from Step (99b) under the conditions reported for the compound of Example 7. The
30 compound was purified by chromatography eluting with 5%

methanol in CH_2Cl_2 to afford 50 mg (21%, 3 steps) of a white powder. MS $(\text{M}+\text{Na})^+ = 488$.

Example 100

5 Binding of Example 7T to cell membranes:

A survey of different cell lines was performed using the radioligand competition binding assay, of the invention, with Example 7T to identify membranes rich in binding sites for Example 7. Cell lines useful for
10 performance of the RCB Assay are preferentially human or mammalian cell lines. It is more preferred that the cell lines express presenilin 1, presenilin 2 and/or presenilin homologs (for example SEL-12). The cell lines surveyed included HEK293 cells (ATCC CRL-1573), IMR 32 (ATCC CCL-
15 127), RAJI (ATCC CCL-86), CHO (ATCC CRL-9096), U-937 (ATCC CRL-1593), and THP-1 (ATCC TIB-202).
Of the cell lines surveyed the best signal to noise ratio (i.e., ratio of specific binding and non-specific binding) was obtained using THP-1 cell membranes.

20

Example 101

Characterization of the Example 11T in the Radioligand Competition Binding Assay:

Example 11, a benzophenone derivative of Example 7,
25 was synthesized. When Example 11 was assayed in the β APPA Assay and separately in the RCB Assay with Example 7T as the radiolabeled standard, a statistically significant correlation of IC_{50} values was observed between the two Assays.

30 Radiolabeled Example 11, i.e. Example 11T, was synthesized and tested in the RCB Assay for equivalency to Example 7T. The apparent K_i was calculated for four compounds (Example 7, Example 11, Example 98, and Example 99) and an statistically significant correlation was
35 observed between results obtained whether the RCB Assay was conducted with Example 7T or Example 11T, indicating that Example 11 binds to the same molecular target(s) in cell membranes. Therefore, it was found that Example 11T could

be used instead of Example 7T as the radioactive tracer in the RCB Assay.

Analogously, it has also been found that Example 43T can be used instead of Example 7T as the radioactive tracer
5 in the RCB Assay.

Example 102

Example 11 reduces the B_{max} of Example 7T:

Cell membranes (THP-1) were incubated with Example 11
10 at approximately 3 times the K_d concentration for 1 hour at room temperature under the conditions outlined for the RCB Assay. Membranes were photolysed at 365nm for 1 hour on ice. Control membranes were incubated in parallel on ice. The membranes were harvested (centrifuged at 40,000G, 4°C,
15 20 minutes) and extensively washed with assay buffer. The membranes were subsequently analyzed in the RCB Assay using Example 7T. A b_{max} of 938fmol/mg membranes was observed for unphotolysed membranes, whereas the b_{max} was reduced to 238fmol/mg membranes after photolysing. However, the K_d
20 for Example 7T was not statistically significantly changed. These results indicate that Example 11 is cross-linked to the membrane binding site of Example 7.

B_{max} is understood by one skilled in the art to represent the maximum number of binding sites in a cell
25 membrane. See Mary Keen (Ed.) Receptor binding techniques. Methods in Molecular Biology, Vol 106, Humana Press, Totowa, New Jersey, 1999.

In this experiment the membranes were photolysed at 365nm, which is appropriate for activation of the
30 benzophenone moiety of Example 11. It is understood that photolysation of the membranes can occur at any wavelength that activates a photoactive tag to cross link to the protein. Such wavelengths generally occur in the 250 to 450 nm range.

35

Example 103

Analysis of cross-linked polypeptides by SDS-PAGE:

THP-1 cell membranes were incubated with Example 11T exactly as outlined under the methods for the RCB Assay of the invention in the presence of an unlabeled competing compound; for Example 98 or Example 99. After 1 hour
5 incubation at room temperature, the membranes were analyzed by the RCB assay (top panel, Figure 1). The membranes in parallel wells were photolysed (365nm, as in Example 102) for 30 minutes on ice (alternatively, at room temperature). Membranes were collected, boiled in SDS-containing buffer
10 in the absence (middle panel Figure 1) or presence of dithiothreitol (50mM) (bottom panel Figure 1) and fractionated by SDS-PAGE (12% acrylamide in the separating gel). The polyacrylamide was fixed in 10% acetic acid/20% methanol/70% water for 45 minutes at room temperature and
15 soaked for another 45 minutes in Amplify™ (Amersham). After drying, the gel was exposed to X-ray film. In the absence of a competing compound, labeling of a number of polypeptides was observed. However, based on the ability of unlabeled compounds to compete with the cross-linking
20 reaction, major polypeptides, that could be specifically cross-linked with Example 11T, of molecular sizes of 30 (band A), 25 (band B), 20 (band C), and 10-12 (band D) kD were identified.

Dose-response experiments using increasing
25 concentrations in the range of 1 picomolar to 100 micromolar of an unlabeled competing compound were performed. The resulting samples were either analyzed by the RCB Assay of the invention prior to photolysis or by SDS-PAGE after photolysis. A statistically significant
30 correlation was observed between the competition in the radioligand competition binding assay and the radioactivity incorporated into the 30 (band A), 25 (band B), 20kD (band C), and 10-12 kD (band D) bands as revealed by SDS-PAGE and fluorography.

35 Thus, the quantitative reduction of cross-linking by unlabeled compounds to the 30, 25, 20, and 10-12 kD bands accurately tracks the reduction of specific binding in the binding assay. These results indicate that identification

of the cross-linked species will identify the site of interaction in the binding assay.

Figure 1 illustrates the correlation between results of the RCB assay and the cross-linking assay for DMSO (lane 1), Example 7 (lane 2), Example 98b (lane 3), Example 43 (lane 4), Example 99 (lane 5), and Example 11 (lane 6) at 1 micromolar. Figure 1, top panel, illustrates results of the RCB Assay for these compounds. Figure 1, middle panel, illustrates results of the cross-linking assay for these compounds under non-reducing conditions. Figure 1, bottom panel, illustrates results of the cross-linking assay for these compounds under reducing conditions. Membranes were incubated with Example 11T and DMSO or a number of unlabeled compounds and analyzed by RCB assay. The total radioactivity associated with the filter off is indicated. Parallel wells were photolysed as in Example 103, and the membrane extracts were analyzed by SDS-PAGE followed by fluorography. The mobility of molecular weight markers (in kD) is indicated to the right. Note specific cross-linking to polypeptides of 30 (band A), 25 (band B), 20 (band C), and 10-12 (band D) polypeptides. The radioactivity associated with band A is stronger than that in bands B to D, suggesting that band A might be a mixture of two polypeptides (i.e., presenilin 1 and presenilin 2; see Figure 4).

Example 104

Immunological identification of the 30kD and 20kD cross-linked polypeptides of Example 103:

THP-1 cell membranes (1mg/ml) in 50mM TRIS buffer, pH 7.4-7.5, were incubated with Example 11T for 1 hour at room temperature and photolysed (as stated above) at room temperature for 30 minutes. The membranes were collected by centrifugation. The membranes were extracted with 50mM Tris, pH 7.5 containing 100mM KCl, 2mM EDTA, 2% CHAPS and 1 complete protease inhibitor tablet per 25 ml buffer (COMPLETE™, Boehringer Mannheim; product number 1697 498)) for 1 hour at 4°C. The detergent soluble fraction was

recovered by centrifugation (40,000 g, 30 min, 4°C). The membrane extract was diluted one half with water. 500ul of the membrane extract were pre-incubated with 10ul of normal mouse IgG and 50ul anti-mouse IgG Sepharose (Sigma) for 1 hour at 4°C. The supernatant was recovered by centrifugation. Subsequently, 10ug of preimmune IgG (Sigma) or 10ug of a monoclonal antibody to presenilin 1 was added in the presence of 50ul anti-mouse IgG Sepharose. Examples of commercially available antibodies to presenilin 1 are Chemicon International: rat anti-human Presenilin 1 monoclonal antibody; product number MAB 1563; or Santa Cruz Biotechnology: goat anti Presenilin 1; product number SC-1244; or Santa Cruz Biotechnology: goat anti Presenilin 1; product number SC-1245. For use with goat antibodies, the immunoprecipitation was altered as follows: normal goat IgG and protein G Sepharose was used for the pre-absorption and protein G Sepharose was used in the presence of the goat antibodies to presenilin 1. The membrane extract was incubated for 5 hours at 4°C. The Sepharose beads were collected by centrifugation and washed 3 times with 25mM Tris, pH 7.5 containing 50mM KCl, 1mM EDTA and 1% CHAPS, followed by 3 washes with phosphate buffered saline. Radioactivity bound to the Sepharose beads was dissociated by boiling in SDS sample buffer (4x) containing 50mM dithiothreitol. The supernatant was loaded onto a 12% SDS-PAGE and the gel was processed as above. Fluorography revealed the presence of the approximately 30 (band A), 20 (band C), and 10kD (band D) polypeptides in the immunoprecipitation with antibodies to presenilin 1, but not with normal mouse IgG. (Figure 2) These results indicate that the membrane binding assay determines, at least in part, the binding of radiolabeled secretase inhibitors to presenilin 1 fragments.

Subsequent experiments established that the lack of polypeptide B in the initial immunoprecipitation experiments was due to aggregation upon boiling of the sample in reduced SDS sample buffer, indicating that all specifically labeled polypeptides (A to D) can be

specifically immunoprecipitated with antibodies to presenilin 1 under non-denaturing conditions.

Figure 2 illustrates a fluorography of a 12% SDS-PAGE. The relative mobility of molecular weight standards (in kD) is indicated to the left. THP-1 membranes were incubated (30 minutes; room temperature) with Example 43T (30nM) alone (panel A) or Example 43T (30nM) in the presence of Example 98 (panel B). The membranes were photolysed at 365nm for 30 minutes and the membranes harvested by centrifugation. The membranes were extracted with 50mM Tris, pH 7.5 containing 100mM KCl, 2mM EDTA, 2% CHAPS in the presence of protease inhibitors for 1 hour at 4°C. The membrane extracts were either directly fractionated by SDS-PAGE (lanes 1 and 6) or after immunoprecipitation with preimmune IgG (lanes 2 and 4) or antibodies to human presenilin 1 (lanes 3 and 5). Note the immunoprecipitation of specifically labeled bands of approximately 30, 20, and 10 kD after cross-linking in the absence of Example 98, but not in the presence of Example 98. The higher molecular weight bands may represent the presenilin 1 holoprotein and/or presenilin 1 aggregates formed in the presence of SDS.

Example 105

Purification of cross-linked polypeptides by affinity chromatography

THP-1 membranes were prepared and cross-linked as in Example 104. The membranes were extracted as in Example 104 at a protein concentration of 10mg membrane protein / 1ml extraction buffer. Normal mouse IgG (Sigma) or monoclonal antibody to the C-terminal loop of presenilin 1 was immobilized on agarose beads at 2mg IgG per 1ml of beads. The membrane extract was diluted one half with water and applied to a normal mouse IgG precolumn, followed by anti-presenilin 1 IgG. The column material was extensively washed with one half diluted extraction buffer, one half diluted extraction buffer containing 1M KCl, and

eluted with 0.1M glycine, pH 2.5 in one half diluted extraction buffer. The resulting polypeptides were analyzed by SDS-PAGE (12% acrylamide in the separating gel), followed by fluorography (left top panel, Figure 3), silver staining (right top panel, Figure 3), immunoblotting using antibodies to the N-terminus of presenilin (left middle panel, Figure 3) or the C-terminus of presenilin 1 (right middle panel, Figure 3). In addition, the silver stain (right top panel, Figure 3) was soaked in Amplify™ (Amersham), dried, and exposed the x-ray film. It is concluded that the specifically cross-linked bands A, B, and C can be enriched by presenilin 1 affinity chromatography. It should be noted that using an antibody to the N-terminus of presenilin 1, also band D could be enriched. Polypeptides A and C are major silver stained protein bands containing the cross-linker of Example 11T and are immunoreactive with antibodies to presenilin 1. It should be noted that the extraction procedure used will not dissociate the association of macromolecules in the presenilin complex. Accordingly, one skilled in the art will understand that this technique can be employed to identify macromolecules associated with the binding site that are involved in beta amyloid precursor processing.

Figure 3 illustrates isolation of cross-linked polypeptides by presenilin 1 affinity chromatography. THP-1 membranes were cross-linked as in Figure 2 and the resulting membrane extracts were applied to a normal mouse IgG Sepharose, followed by an anti-presenilin 1 Sepharose. The starting material (lanes 1), flow-through normal mouse IgG (lanes 2), flow-through presenilin 1 Sepharose (lanes 3), last wash prior to elution (lanes 4), and elution by lowering the pH (lanes 5) are indicated. The relative mobility of molecular weight markers is indicated. The left top panel shows a fluorography of a 12% SDS-PAGE. Note the enrichment of bands A to C on the presenilin 1 affinity column. The silver stain (top right panel) reveals that bands A and C are clearly enriched,

distinguishable from contaminating proteins, and present in purity sufficient for sequence analysis. The silver stain was soaked in Amplify™ (Amersham), dried, and exposed to x-ray film (bottom left panel). It should be noted that major polypeptides in the elution fraction as revealed by silver staining perfectly align with the radioactivity as revealed by fluorography. The identify of band A as presenilin 1 N-terminal fragments was revealed by immunoblotting using N-terminal-specific antibodies (left middle panel), whereas band C was identified as presenilin C-terminal fragments (right middle panel).

It is understood by one skilled in the art that this or similar purification schemes can be employed to isolate radiolabeled binding polypeptides in sufficient quantities to allow for N-terminal amino acid or mass spectroscopy analysis. Also, one skilled in the art understands that isolated radiolabeled polypeptides can be further fractionated after chemical or proteolytic digestion to isolate one or several radiolabeled polypeptides in the sizes of approximately 2 to 100 amino acids. Sequence analysis will reveal the location of the smaller polypeptides in the protein sequence of the binding site molecules. In addition, this method can be used to define specifically cross-linked amino acids in the binding site. This information can ultimately be used in rational drug design for Alzheimer's disease. It should be noted that both N- and C-terminal presenilin 1 fragments are labeled by Example 11T. This observation is consistent with the notion that the binding site is contained in proteolytic fragments of presenilin 1 generated upon incorporation in the presenilin 1 complex.

Example 106

35 Evidence for the involvement of presenilin 2 in the binding site

THP-1 membranes were prepared and analyzed as in Example 104. The presenilin 1 antibodies were replaced

with rabbit polyclonal antibodies specific for presenilin 2. The following modifications were included in comparison to Example 104: samples were pre-absorbed with normal rabbit IgG and protein A Sepharose was used instead of anti-mouse IgG Sepharose. The resulting immunoprecipitates were analyzed by SDS-PAGE (12% acrylamide in the separating gel) followed by fluorography. (See Figure 4) Bands A and B was specifically precipitated with an antibody to presenilin 2 (N-terminus), whereas antibodies to the C-terminus of presenilin 2 preferentially identified band B. These results indicate that the membrane binding assay determines, at least in part, the binding of radiolabeled secretase inhibitors to presenilin 2 fragments. One skilled in the art will realize that the cross-linking assay can be used to identify compounds with preferential affinity for either presenilin 1 or 2. Membranes derived from organisms lacking either presenilin 1 or 2, or both, might be used for the same purpose.

Figure 4 illustrates a fluorography of a 12% SDS-PAGE. The relative mobility of molecular weight standards (in kD) is indicated to the right. THP-1 membranes were incubated (30 minutes; room temperature) with Example 11T, photolyzed at 365nm for 30 minutes, and the membranes harvested by centrifugation. The membranes were extracted with 50mM Tris, pH 7.5 containing 100mM KCl, 2mM EDTA, 2% CHAPS in the presence of protease inhibitors for 1 hour at 4°C. The membrane extracts were either directly fractionated by SDS-PAGE (lane 1) or after immunoprecipitation with preimmune IgG (lane 4) or antibodies to human presenilin 2 (lane 2, PS-2 N-terminal specific antibody; lane 3, PS-2 C-terminal specific antibody). Note the immunoprecipitation of specifically labeled bands A and B of approximately 30 and 25 kD.

It is understood by one skilled in the art that the assays disclosed herein, specifically the Radio Competition Binding Assay and the cross-linking Assay may be employed to differentiate between inhibitors specific for

presenilin-1 and presenilin-2. For example, differential competition for radioactivity incorporation in bands A to D would indicate presenilin-1 and/or presenilin-2 specific compounds. Moreover, binding to membranes derived from mammalian cells deficient in either PS-1 or PS-2 may be employed to identify PS-1 or PS-2 specific compounds. For example these cells may be derived from organisms, for example murine, which are gene targeted for PS-1 or PS-2. Examples of cells include fibroblasts, neurons, and whole embryonic membranes.

It is understood that the isolation and sequence data for presenilin-1 (PS-1) cloning has been published in Sherrington R et al., Nature, Vol 375, pp754-760, 1995, herein incorporated by reference. It is also understood that the isolation and sequence data for presenilin-2 (PS-2) cloning has been published in Rogaev E.I. et al., Nature, Vol 376, pp774-778, 1995, herein incorporated by reference.

In Vivo Diagnostic Imaging Utility

The radiolabeled compounds of the invention are useful as radiopharmaceuticals for imaging sites involved in beta-amyloid production, and thus may be used to diagnose present or potential disorders involving beta-amyloid production, including but not limited to Alzheimer's disease. The patient may be any type of a mammal, but is preferably a human. The radiolabeled compounds may be used alone, or may be employed as a composition with a radiopharmaceutically acceptable carrier, and/or in combination with other diagnostic or therapeutic agents. Suitable radiopharmaceuticals carriers and suitable amounts thereof are well known in the art, and can be found in, for example, Remington's Pharmaceutical Sciences, Gennaro, A.R., ed., Mack Publishing Company, Easton, PA (1985), and The United States Pharmacopia - The National Formulary, 22nd Revision, Mack Printing Company, Easton, PA (1990),

standard reference texts in the pharmaceutical field. Other materials may be added, as convenient, to stabilize the composition, as those skilled in the art will recognize, including antioxidizing agents such as sodium
5 bisulfite, sodium sulfite, ascorbic acid, gentisic acid or citric acid (or their salts) or sodium ethylenediamine tetraacetic acid (sodium EDTA), as is well known in the art. Such other materials, as well as suitable amounts thereof, are also described in Remington's Pharmaceutical
10 Sciences and The United States Pharmacopia - The National Formulary, cited above.

The present invention also includes radiopharmaceutical kits containing the labeled compounds of the invention. Such kits may contain the labeled
15 compounds in sterile lyophilized form, and may include a sterile container of a radiopharmaceutically acceptable reconstitution liquid. Suitable reconstitution liquids are disclosed in Remington's Pharmaceutical Sciences and The United States Pharmacopia - The National Formulary, cited
20 above. Such kits may alternatively contain a sterile container of a composition of the radiolabeled compounds of the invention. Such kits may also include, if desired, other conventional kit components, such as, for example, one or more carriers, one or more additional vials for
25 mixing. Instructions, either as inserts or labels, indicating quantities of the labeled compounds of the invention and carrier, guidelines for mixing these components, and protocols for administration may also be included in the kit. Sterilization of the containers and
30 any materials included in the kit and lyophilization (also referred to as freeze-drying) of the labeled compounds of the invention may be carried out using conventional sterilization and lyophilization methodologies known to those skilled in the art.

35 To carry out the method of the invention, the radiolabeled compounds are generally administered intravenously, by bolus injection, although they may be administered by any means that produces contact of the

compounds with sites of beta-amyloid production, particularly in sites in the brain. Suitable amounts for administration will be readily ascertainable to those skilled in the art, once armed with the present disclosure.

5 The dosage administered will, of course, vary depending up such known factors as the particular compound administered, the age, health and weight or the nature and extent of any symptoms experienced by the patient, the amount of radiolabeling, the particular radionuclide used as the

10 label, the rate of clearance of the radiolabeled compounds from the patient.

Acceptable ranges for administration of radiolabeled materials are tabulated, for example, in the Physicians Desk Reference (PDR) for Nuclear Medicine, published by

15 Medical Economics Company, a well-known reference text. A discussion of some of the aforementioned considerations is provided in Eckelman et al., J. Nucl. Med., Vol. 209, pp. 350-357 (1979). By way of general guidance, a dosage range of the radiolabeled compounds of the invention may be

20 between about 1 and about 40 mCi.

Once the radiolabeled compounds of the invention are administered, the presence of sites involved in beta-amyloid production may be visualized using standard imaging systems. Such imaging systems are well known in

25 the art, and are discussed, for example, in Macovski, A., Medical Imaging Systems, Information and Systems Science Series, Kailath, T., ed., Prentice-Hall, Inc., Englewood Cliffs, NJ (1983). Particularly preferred is positron emission tomography (PET). Specifically, imaging is

30 carried out by scanning the entire patient, or a particular region of the patient using the detection system, and detecting the radioisotope signal. The detected signal is then converted into an image. The resultant images should be read by an experienced observer, such as, for example, a

35 nuclear medicine physician. The foregoing process is referred to herein as "imaging" the patient. Generally, imaging is carried out about 1 minute to about 48 hours following administration of the radiolabeled compound of

the invention. The precise timing of the imaging will be dependant upon such factors as the half-life of the radioisotope employed, and the clearance rate of the compound administered, as will be readily apparent to those skilled in the art. Preferably, imaging is carried out between about 1 minute and about 4 hours following administration.

The advantage of employing the radiolabeled compounds of the invention, which have the ability to localize specifically and with high affinity in sites involved in beta-amyloid production, to detect the presence of such sites involved in beta-amyloid production and/or to diagnose disorders in a patient involving beta-amyloid production, will be readily apparent to those skilled in the art, once armed with the present disclosure.

Dosage and Formulation

The compounds determined from the present invention can be administered orally using any pharmaceutically acceptable dosage form known in the art for such administration. The active ingredient can be supplied in solid dosage forms such as dry powders, granules, tablets or capsules, or in liquid dosage forms, such as syrups or aqueous suspensions. The active ingredient can be administered alone, but is generally administered with a pharmaceutical carrier. A valuable treatise with respect to pharmaceutical dosage forms is Remington's Pharmaceutical Sciences, Mack Publishing.

The compounds determined from the present invention can be administered in such oral dosage forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. Likewise, they may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular form, all using dosage forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be

employed to prevent or treat neurological disorders related to β -amyloid production or accumulation, such as Alzheimer's disease and Down's Syndrome.

5 The compounds of this invention can be administered by any means that produces contact of the active agent with the agent's site of action in the body of a host, such as a human or a mammal. They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or
10 in a combination of therapeutic agents. They can be administered alone, but generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

15 The dosage regimen for the compounds determined from the present invention will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the species, age, sex, health, medical
20 condition, and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; the route of administration, the renal and hepatic function of the patient, and the effect desired. An ordinarily skilled physician or
25 veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

Advantageously, compounds determined from the present invention may be administered in a single daily dose, or
30 the total daily dosage may be administered in divided doses of two, three, or four times daily.

The compounds identified using the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes,
35 using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage

administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

In the methods of the present invention, the compounds herein described in detail can form the active ingredient, and are typically administered in admixture with suitable pharmaceutical diluents, excipients, or carriers (collectively referred to herein as carrier materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents, and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or β -lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth, or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum, and the like.

The compounds determined from the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles.

Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

Compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such
5 polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide-phenol, polyhydroxyethylaspartamidephenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues.
Furthermore, the compounds determined from the present
10 invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters,
15 polyacetals, polydihdropyrans, polycyanoacylates, and crosslinked or amphipathic block copolymers of hydrogels.

Gelatin capsules may contain the active ingredient and powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the
20 like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any
25 unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract.

Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

30 In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water
35 soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable

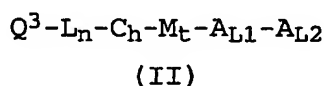
stabilizing agents. Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

- 5 Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field.

CLAIMS

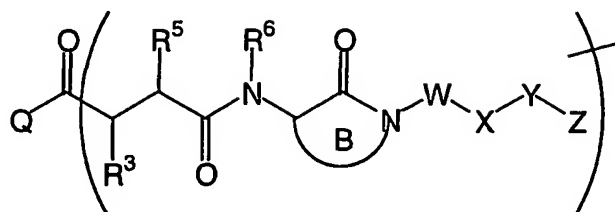
What is claimed is:

- 5 1. A method for diagnosing a neurological disease involving APP processing and/or the production of beta-amyloid production within a mammalian body comprising:
- (c) administering a diagnostically effective amount of a radiopharmaceutical inhibitor of APP processing and/or
10 the production of beta-amyloid production; and
- (d) imaging the area of the patient wherein the disease is located.
2. The method of Claim 1 wherein the radiopharmaceutical
15 comprises technetium-99m, indium-111, or gallium-68.
3. The method of Claim 1 wherein the radiopharmaceutical comprises technetium-99m.
- 20 4. The method of Claim 1 wherein the radiopharmaceutical is a compound of Formula II:



- 25 wherein
- Q^3 is an inhibitor of APP processing and/or an inhibitor of beta-amyloid production;
- L_n is a linking group;
- C_h is a radionuclide metal chelator coordinated to a
30 transition metal radionuclide M_t ;
- M_t is a transition metal radionuclide;
- A_{L1} is a first ancillary ligand; and
- A_{L2} is a second ancillary ligand capable of stabilizing the radiopharmaceutical;
- 35 and pharmaceutically acceptable salts thereof.

5. The method of Claim 4 wherein Q^3 is a radical of a compound of formula (I):



(I)

wherein:

Q is $-NH_2$;

R^3 is C_1 - C_6 alkyl substituted with 0-1 R^4 ;

R^4 is H, OH, C_1 - C_6 alkoxy, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 -
10 C_6 alkynyl, C_3 - C_{10} carbocycle, C_6 - C_{10} aryl, or 5 to 10
membered heterocycle;

R^5 is H, OR^{14} ;

C_1 - C_6 alkyl substituted with 0-3 R^{5b} ;

C_1 - C_6 alkoxy substituted with 0-3 R^{5b} ;

15 C_2 - C_6 alkenyl substituted with 0-3 R^{5b} ;

C_2 - C_6 alkynyl substituted with 0-3 R^{5b} ;

C_3 - C_{10} carbocycle substituted with 0-3 R^{5c} ;

C_6 - C_{10} aryl substituted with 0-3 R^{5c} ; or

5 to 10 membered heterocycle substituted with 0-3 R^{5c} ;

20 R^{5b} , at each occurrence, is independently selected from:

H, C_1 - C_6 alkyl, CF_3 , OR^{14} , Cl, F, Br, I, =O, CN, NO_2 ,
 $NR^{15}R^{16}$;

C_3 - C_{10} carbocycle substituted with 0-3 R^{5c} ;

C_6 - C_{10} aryl substituted with 0-3 R^{5c} ; or

25 5 to 10 membered heterocycle substituted with 0-3 R^{5c} ;

R^{5c} , at each occurrence, is independently selected from H,
OH, C_1 - C_6 alkyl, C_1 - C_4 alkoxy, Cl, F, Br, I, CN, NO_2 ,
 $NR^{15}R^{16}$, or CF_3 ;

R^6 is H;

30 C_1 - C_6 alkyl substituted with 0-3 R^{6a} ;

C_3 - C_{10} carbocycle substituted with 0-3 R^{6b} ; or

C_6 - C_{10} aryl substituted with 0-3 R^{6b} ;

- R^{6a} , at each occurrence, is independently selected from H, C₁-C₆ alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶, phenyl or CF₃;
- R^{6b} , at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;
- W is $-(CR^8R^{8a})_p-$;
- p is 0 to 4;
- R^8 and R^{8a} , at each occurrence, are independently selected from H, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₂-C₄ alkynyl and C₃-C₈ cycloalkyl;
- X is a bond;
- C₆-C₁₀ aryl substituted with 0-3 R^{Xb} ;
- C₃-C₁₀ carbocycle substituted with 0-3 R^{Xb} ; or
- 5 to 10 membered heterocycle substituted with 0-3 R^{Xb} ;
- R^{Xb} , at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;
- Y is a bond or $-(CR^9R^{9a})_t-V-(CR^9R^{9a})_u-$;
- t is 0 to 3;
- u is 0 to 3;
- R^9 and R^{9a} , at each occurrence, are independently selected from H, C₁-C₆ alkyl or C₃-C₈ cycloalkyl;
- V is a bond, $-C(=O)-$, $-O-$, $-S-$, $-S(=O)-$, $-S(=O)_2-$, $-N(R^{19})-$, $-C(=O)NR^{19b}-$, $-NR^{19b}C(=O)-$, $-NR^{19b}S(=O)_2-$, $-S(=O)_2NR^{19b}-$, $-NR^{19b}S(=O)-$, $-S(=O)NR^{19b}-$, $-C(=O)O-$, or $-OC(=O)-$;
- Z is H;
- C₁-C₈ alkyl substituted with 0-2 R^{12} ;
- C₂-C₄ alkenyl substituted with 0-2 R^{12} ;
- C₂-C₄ alkynyl substituted with 0-2 R^{12} ;
- C₆-C₁₀ aryl substituted with 0-4 R^{12b} ;
- C₃-C₁₀ carbocycle substituted with 0-4 R^{12b} ; or
- 5 to 10 membered heterocycle substituted with 0-3 R^{12b} ;
- R^{12} is C₆-C₁₀ aryl substituted with 0-4 R^{12b} ;
- C₃-C₁₀ carbocycle substituted with 0-4 R^{12b} ; or

- 5 to 10 membered heterocycle substituted with 0-3
R^{12b};
- R^{12b}, at each occurrence, is independently selected from H,
OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂,
5 NR¹⁵R¹⁶, or CF₃;
- B is a 5 to 10 membered lactam, wherein the lactam is
saturated, partially saturated or unsaturated; wherein
each additional lactam carbon is substituted with 0-2
R¹¹; and, optionally, the lactam contains a heteroatom
10 selected from -O-, -S-, -S(=O)-, -S(=O)₂-, -N= and -
N(R¹⁰)-;
- R¹⁰ is H, C(=O)R¹⁷, C(=O)OR¹⁷, C(=O)NR¹⁸R¹⁹,
S(=O)₂NR¹⁸R¹⁹, S(=O)₂R¹⁷;
C₁-C₆ alkyl optionally substituted with R^{10a};
15 C₆-C₁₀ aryl substituted with 0-4 R^{10b};
C₃-C₁₀ carbocycle substituted with 0-3 R^{10b}; or
5 to 10 membered heterocycle optionally substituted
with 0-3 R^{10b};
- R^{10a}, at each occurrence, is independently selected from H,
20 C₁-C₆ alkyl, C₃-C₆ cycloalkyl, OR¹⁴, Cl, F, Br, I, =O,
CN, NO₂, NR¹⁵R¹⁶, phenyl or CF₃;
- R^{10b}, at each occurrence, is independently selected from H,
OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂,
NR¹⁵R¹⁶, or CF₃;
- 25 R¹¹ is C₁-C₄ alkoxy, Cl, F, Br, I, =O, CN, NO₂, NR¹⁸R¹⁹,
C(=O)R¹⁷, C(=O)OR¹⁷, C(=O)NR¹⁸R¹⁹, S(=O)₂NR¹⁸R¹⁹, CF₃;
C₁-C₆ alkyl optionally substituted with R^{11a};
C₆-C₁₀ aryl substituted with 0-3 R^{11b};
C₃-C₁₀ carbocycle substituted with 0-3 R^{11b}; or
30 5 to 10 membered heterocycle substituted with 0-3 R^{11b};
alternatively, two R¹¹ substituents on the same carbon
atoms may be combined to form a C₃-C₆ carbocycle;
alternatively, two R¹¹ substituents on adjacent carbon
atoms may be combined to form a C₃-C₆ carbocycle or a
35 benzo fused radical, wherein said benzo fused radical
is substituted with 0-3 R¹³;

- R^{11a}, at each occurrence, is independently selected from H, C₁-C₆ alkyl, OR¹⁴, Cl, F, Br, I, =O, CN, NO₂, NR¹⁵R¹⁶, phenyl or CF₃;
- 5 R^{11b}, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;
- R¹³, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, C₁-C₄ alkoxy, Cl, F, Br, I, CN, NO₂, NR¹⁵R¹⁶, or CF₃;
- 10 R¹⁴ is H, phenyl, benzyl, C₁-C₆ alkyl, or C₂-C₆ alkoxyalkyl;
- R¹⁵, at each occurrence, is independently selected from H, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);
- 15 R¹⁶, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);
- R¹⁷ is H, phenyl, benzyl, C₁-C₆ alkyl, or C₂-C₆ alkoxyalkyl;
- 20 R¹⁸, at each occurrence, is independently selected from H, C₁-C₆ alkyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl); and
- R¹⁹, at each occurrence, is independently selected from H, OH, C₁-C₆ alkyl, phenyl, benzyl, phenethyl, -C(=O)-(C₁-C₆ alkyl) and -S(=O)₂-(C₁-C₆ alkyl);
- 25 R^{19b} is H, C₁-C₆ alkyl, C₃-C₈ cycloalkyl, phenyl, benzyl or phenethyl; and
- R²⁰ is H or C₁-C₆ alkyl.
- 30 6. The method of Claim 4 wherein Q³ is an inhibitor of beta-amyloid production selected from the group consisting of:
- 35 (1) an inhibitor of presenilin-1;
- (2) an inhibitor of presenilin-2;
- (3) an inhibitor of β secretase;
- (4) an inhibitor of α secretase;
- (5) an inhibitor of γ secretase; and
- (6) an inhibitor of BACE/memapsin 2.

7. The method of Claim 4 wherein Q^3 is an inhibitor of beta-amyloid production which is identified by the method of screening for inhibitors of beta-amyloid production

5 comprising,

- 1) contacting a potential inhibitor of beta-amyloid production and a tagged inhibitor of beta-amyloid production with at least one macromolecule involved in the processing of APP and the production of beta-amyloid peptide, said macromolecule containing a binding site specific for said tagged inhibitor of beta-amyloid production;
- 10 2) separating the tagged inhibitor of beta-amyloid production bound to said macromolecule from the tagged inhibitor of beta-amyloid production free from said macromolecule; and
- 15 3) determining an inhibitory concentration of the potential inhibitor of beta-amyloid production from the concentration of tagged inhibitor of beta-amyloid production bound to said macromolecule.
- 20

8. The method of Claim 4 wherein L_n is a linking group of about 5 Angstroms to about 10,000 Angstroms in length.

25 9. The method of Claim 4 wherein L_n is a linking group of the formula $-M^{31}-Y^{31}(CR^{31}R^{32})_f(Z^1)_fY^{32}-M^{32}-$;

wherein

M^{31} is $-(CH_2)_gZ^{31}]_{g'}-(CR^{31}R^{32})_{g''}-$;

M^{32} is $-(CR^{31}R^{32})_{g''}-[Z^{31}(CH_2)_g]_{g'}-$;

30 g is independently 0-10;

g' is independently 0-1;

g'' is independently 0-10;

f is independently 0-10;

f' is independently 0-10;

35 f'' is independently 0-1;

Y^{31} and Y^{32} , at each occurrence, are independently selected from: a direct bond, $-O-$, $-NR^{32}-$, $-C(=O)-$, $-C(=O)O-$, $-$

OC(=O)O-, -C(=O)NH-, -C(=NR³²)-, -S-, -SO-, -SO₂-, -SO₃-, -NHC(=O)-, -(NH)₂C(=O)-, -(NH)₂C=S-;

5 Z³¹ is independently selected at each occurrence from a (C₆-C₁₄) saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R³³; and a heterocyclic ring system, optionally substituted with 0-4 R³³;

R³¹ and R³² are independently selected at each occurrence from: hydrogen; (C₁-C₁₀)alkyl substituted with 0-5 R³³;

10 alkaryl wherein the aryl is substituted with 0-5 R³³;

R³³ is independently selected at each occurrence from the group: hydrogen, -OH, -NHR³⁴, -C(=O)R³⁴, -OC(=O)R³⁴, -OC(=O)OR³⁴, -C(=O)OR³⁴, -C(=O)NR³⁴, -CN, -SR³⁴, -SOR³⁴, -SO₂R³⁴, -NHC(=O)R³⁴, -NHC(=O)NHR³⁴, or -NHC(=S)NHR³⁴;

15 and

R³⁴ is independently selected at each occurrence from the group: hydrogen; (C₁-C₆)alkyl; benzyl, and phenyl.

10. The method of Claim 4 wherein:

20 L_n is a linking group of the formula -R³⁵-G-R³⁶-;

R³⁵ and R³⁶ are each independently -N(R³⁷)C(=O)-, -C(=O)N(R³⁷)-, -OC(=O)-, -C(=O)O-, -O-, -S-, -S(O)-, -SO₂-, -NR³⁷-, -C(=O)-, or a direct bond;

each R³⁷ is independently H or (C₁-C₆)alkyl;

25 G is (C₁-C₂₄)alkyl substituted with 0-3 R³⁸, cycloalkyl substituted with 0-3 R³⁸, aryl substituted with 0-3 R³⁸, or heterocycle substituted with 0-3 R³⁸;

30 R³⁸ is =O, F, Cl, Br, I, -CF₃, -CN, -CO₂R³⁹, -C(=O)R³⁹, -C(=O)N(R³⁹)₂, -CHO, -CH₂OR³⁹, -OC(=O)R³⁹, -OC(=O)OR⁴⁰, -OR³⁹, -OC(=O)N(R³⁹)₂, -NR³⁹C(=O)R³⁹, -NR⁴¹C(=O)OR⁴⁰, -NR³⁹C(=O)N(R³⁹)₂, -NR³⁹SO₂N(R³⁹)₂, -NR⁴¹SO₂R⁴⁰, -SO₃H, -SO₂R⁴⁰, -SR³⁹, -S(=O)R⁴⁰, -SO₂N(R³⁹)₂, -N(R³⁹)₂, -NHC(=NH)NHR³⁹, -C(=NH)NHR³⁹, =NOR³⁹, -NO₂, -C(=O)NHOR³⁹, -C(=O)NHN(R³⁹)R⁴⁰, or -OCH₂CO₂H;

35 R³⁹, R⁴⁰, and R⁴¹ are each independently selected at each occurrence from the group: a direct bond, H, and (C₁-C₆)alkyl.

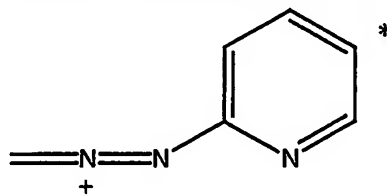
11. The method of Claim 4 wherein:

C_h is selected from the group: $-R^{42}N=N^+=$, $-R^{42}R^{43}N-N=$, $-R^{42}N=$, and $-R^{42}N=N(H)-$, wherein

- 5 R^{42} is a direct bond, (C_1-C_{10}) alkyl substituted with 0-3 R^{44} , aryl substituted with 0-3 R^{44} , cycloalkyl substituted with 0-3 R^{44} , heterocycle substituted with 0-3 R^{44} , heterocycloalkyl substituted with 0-3 R^{44} , aralkyl substituted with 0-3 R^{44} , or alkaryl substituted with 0-3 R^{44} ;
- 10 R^{43} is hydrogen, aryl substituted with 0-3 R^{44} , (C_1-C_{10}) alkyl substituted with 0-3 R^{44} , and a heterocycle substituted with 0-3 R^{44} ;
- R^{44} is a direct bond, $=O$, F , Cl , Br , I , $-CF_3$, $-CN$, $-CO_2R^{45}$, $-C(=O)R^{45}$, $-C(=O)N(R^{45})_2$, $-CHO$, $-CH_2OR^{45}$, $-OC(=O)R^{45}$, $-OC(=O)OR^{46}$, $-OR^{45}$, $-OC(=O)N(R^{45})_2$, $-NR^{45}C(=O)R^{45}$, $-NR^{47}C(=O)OR^{46}$, $-NR^{45}C(=O)N(R^{45})_2$, $-NR^{45}SO_2N(R^{45})_2$, $-NR^{47}SO_2R^{46}$, $-SO_3H$, $-SO_2R^{46}$, $-SR^{45}$, $-S(=O)R^{46}$, $-SO_2N(R^{45})_2$, $-N(R^{45})_2$, $-NHC(=NH)NHR^{45}$, $-C(=NH)NHR^{45}$, $=NOR^{45}$, NO_2 , $-C(=O)NHOR^{45}$, $-C(=O)NHN(R^{45})R^{46}$, or $-OCH_2CO_2H$;
- 15 R^{45} , R^{46} , and R^{47} are each independently selected at each occurrence from the group: a direct bond, H , and (C_1-C_6) alkyl.

25

12. The method of Claim 4 wherein C_h is



and is attached to L_n at the carbon designated with a *.

30

13. The method of Claim 4 wherein M_t is technetium-99m.

14. The method of Claim 4 wherein M_t is rhenium-186.

15. The method of Claim 4 wherein M_t is rhenium-188.

16. The method of Claim 4 wherein A_{L1} is a halide, a dioxygen ligand, or a functionalized aminocarboxylate.

5

17. The method of Claim 4 wherein A_{L1} is tricine.

18. The method of Claim 4 wherein:

A_{L2} is selected from the group: $-A^{31}$ and $-A^{32}-W-A^{33}$;

10 A^{31} is $-PR^{91}R^{92}R^{93}$ or $-AsR^{91}R^{92}R^{93}$;

A^{32} and A^{33} are each independently $-PR^{91}R^{92}$ or $-AsR^{91}R^{92}$;

W is a spacer group selected from the group: (C_1-C_{10}) alkyl

substituted with 0-3 R^{94} , aryl substituted with 0-3

R^{94} , cycloalkyl substituted with 0-3 R^{94} , heterocycle

15 substituted with 0-3 R^{94} , heterocycloalkyl substituted

with 0-3 R^{94} , aralkyl substituted with 0-3 R^{94} and

alkaryl substituted with 0-3 R^{94} ;

R^{91} , R^{92} , and R^{93} are independently selected at each

occurrence from the group: (C_1-C_{10}) alkyl substituted

20 with 0-3 R^{94} , aryl substituted with 0-3 R^{94} , cycloalkyl

substituted with 0-3 R^{94} , heterocycle substituted with

0-3 R^{94} , aralkyl substituted with 0-3 R^{94} , alkaryl

substituted with 0-3 R^{94} , and arylalkaryl substituted

with 0-3 R^{94} ;

25 R^{94} is independently selected at each occurrence from the

group: F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{95}$, $-C(=O)R^{95}$, -

$C(=C)N(R^{95})_2$, $-CH_2OR^{95}$, $-OC(=O)R^{95}$, $-OC(=O)OR^{96}$, $-OR^{95}$,

$-OC(=O)N(R^{95})_2$, $-NR^{95}C(=O)R^{95}$, $-NR^{95}C(=O)OR^{95}$, -

$NR^{95}C(=O)N(R^{95})_2$, SO_3^- , $-NR^{95}SO_2N(R^{95})_2$, $-NR^{95}SO_2R^{96}$, -

30 SO_3H , $-SO_2R^{95}$, $-S(=O)R^{95}$, $-SO_2N(R^{95})_2$, $-N(R^{95})_2$, -

$N(R^{95})_3^+$, $-NHC(=NH)NHR^{95}$, $-C(=NH)NHR^{95}$, $=NOR^{95}$, $-NO_2$, -

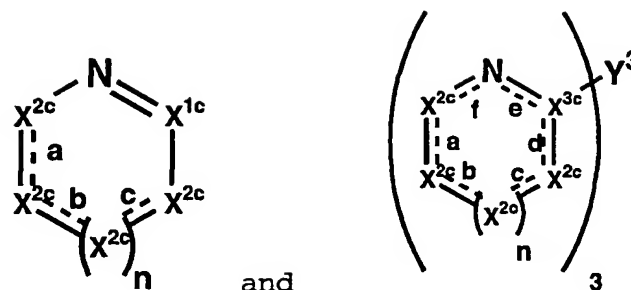
$C(=O)NHOR^{95}$, $-C(=O)NHNHR^{95}R^{96}$, and $-OCH_2CO_2H$; and

R^{95} and R^{96} are independently selected at each occurrence

from the group: hydrogen and (C_1-C_6) alkyl.

35

19. The method of Claim 4 wherein A_{L2} is an ancillary ligand selected from the group:



wherein

n is 0 or 1;

X^{1c} is independently selected at each occurrence from the
 5 group: CR^{84} and N;

X^{2c} is independently selected at each occurrence from the
 group: CR^{84} , $CR^{84}R^{84}$, N, NR^{84} , O and S;

X^{3c} is independently selected at each occurrence from the
 group: C, CR^{84} , and N;

10 provided the total number of heteroatoms in each ring of
 the ligand Al_2 is 1 to 4;

Y^3 is selected from the group: BR^{84-} , CR^{84} , (P=O), (P=S);
 and a, b, c, d, e and f indicate the positions of optional
 double bonds, provided that one of e and f is a double
 15 bond;

R^{64} is independently selected at each occurrence from the
 group:

H, (C₁-C₁₀)alkyl substituted with 0-3 R^{85} , (C₂-
 C₁₀)alkenyl substituted with 0-3 R^{85} , (C₂-
 20 C₁₀)alkynyl substituted with 0-3 R^{85} , aryl
 substituted with 0-3 R^{85} , carbocycle substituted
 with 0-3 R^{85} , and R^{85} ;

or, alternatively, two R^{84} may be taken together with
 the atom or atoms to which they are attached to
 25 form a fused aromatic, carbocyclic or
 heterocyclic ring, substituted with 0-3 R^{85} ;

R^{85} is independently selected at each occurrence from
 the group: =O, F, Cl, Br, I, -CF₃, -CN, -NO₂,
 -CO₂ R^{86} , -C(=O) R^{86} , -C(=O)N(R^{86})₂, -N(R^{86})₃⁺
 30 -CH₂OR⁸⁶, -OC(=O) R^{86} , -OC(=O)OR^{86a}, -OR⁸⁶,
 -OC(=O)N(R^{86})₂, -NR⁸⁶C(=O) R^{86} , -NR⁸⁷C(=O)OR^{86a},
 -NR⁸⁶C(=O)N(R^{86})₂, -NR⁸⁷SO₂N(R^{86})₂, -NR⁸⁷SO₂R^{86a},

-SO₃H, -SO₂R^{86a}, -SO₂N(R⁸⁶)₂, -N(R⁸⁶)₂, -OCH₂CO₂H;

and

R⁸⁶, R^{86a}, and R⁸⁷ are each independently selected at each occurrence from the group: hydrogen and (C₁-C₆)alkyl.

20. The method of Claim 4 wherein A_{L2} is -PR⁴⁸R⁴⁹R⁵⁰.

21. The method of Claim 20 wherein R⁴⁸, R⁴⁹, and R⁵⁰ are each aryl substituted with one R⁵¹ substituent.

22. The method of Claim 21 wherein each aryl is phenyl.

23. The method of Claim 21 wherein each R⁵¹ substituent is SO₃H or SO₃⁻, in the meta position.

24. The method of Claim 4 wherein the radiopharmaceutical is a compound of Formula V:



wherein

Q³ is an inhibitor of APP processing and/or the production of beta-amyloid production;

L_n is a linking group of the formula -M³¹-Y³¹(CR³¹R³²)_f(Z¹)_{f'}Y³²-M³²-;

wherein

M³¹ is -[(CH₂_gZ³¹)_{g'}-(CR³¹R³²)_{g''}]-;

M³² is -(CR³¹R³²)_{g''}-[Z³¹(CH₂)_g]_{g'}-;

g is independently 0-10;

g' is independently 0-1;

g'' is independently 0-10;

f is independently 0-10;

f' is independently 0-10;

f'' is independently 0-1;

Y³¹ and Y³², at each occurrence, are independently selected from: a direct bond, -O-, -NR³²-, -C(=O)-, -C(=O)O-, -

OC(=O)O-, -C(=O)NH-, -C(=NR³²)-, -S-, -SO-, -SO₂-, -
SO₃-, -NHC(=O)-, -(NH)₂C(=O)-, -(NH)₂C=S-;

5 Z³¹ is independently selected at each occurrence from a (C₆-
C₁₄) saturated, partially saturated, or aromatic
carbocyclic ring system, substituted with 0-4 R³³; and
a heterocyclic ring system, optionally substituted
with 0-4 R³³;

10 R³¹ and R³² are independently selected at each occurrence
from: hydrogen; (C₁-C₁₀)alkyl substituted with 0-5 R³³;
alkaryl wherein the aryl is substituted with 0-5 R³³;

R³³ is independently selected at each occurrence from the
group: hydrogen, -OH, -NHR³⁴, -C(=O)R³⁴, -OC(=O)R³⁴, -
OC(=O)OR³⁴, -C(=O)OR³⁴, -C(=O)NR³⁴, -CN, -SR³⁴, -SOR³⁴,
-SO₂R³⁴, -NHC(=O)R³⁴, -NHC(=O)NHR³⁴, or -NHC(=S)NHR³⁴;

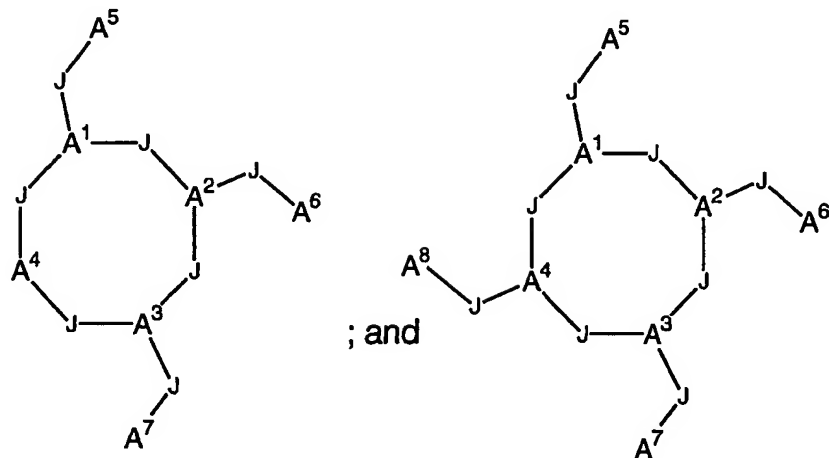
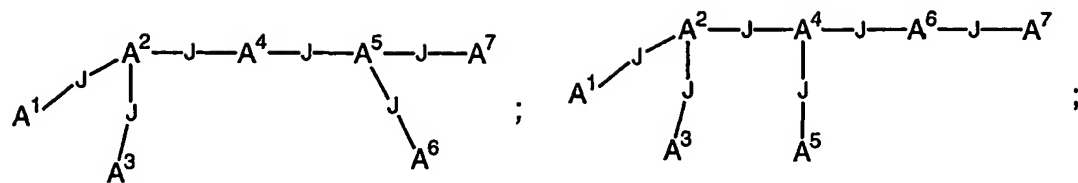
15 and

R³⁴ is independently selected at each occurrence from the
group: hydrogen; (C₁-C₆)alkyl; benzyl, and phenyl;

C_h is a radionuclide metal chelator coordinated to a
transition metal radionuclide M_t;

20 M_t is a transition metal radionuclide;
and pharmaceutically acceptable salts thereof.

25. The method of Claim 24 wherein C_h is selected from
the group:



wherein:

A¹, A², A³, A⁴, A⁵, A⁶, and A⁷ are independently

- 5 selected at each occurrence from the group:
 NR⁶⁰R⁶¹, S, SH, S(Pg), O, OH, PR⁶²R⁶³, P(O)R⁶²R⁷³,
 P(S)R⁶²R⁶³, P(NR⁶⁷)R⁶²R⁶³;

- J is a direct bond, CH, or a spacer group selected
 10 from the group: (C₁-C₁₀)alkyl substituted with 0-3
 R⁷², aryl substituted with 0-3 R⁷², cycloalkyl
 substituted with 0-3 R⁷², heterocycloalkyl
 substituted with 0-3 R⁷², aralkyl substituted
 with 0-3 R⁷² and alkaryl substituted with 0-3 R⁷²;
 15 R⁶⁰, R⁶¹, R⁶², R⁶³, and R⁶⁴ are each independently
 selected from the group: a direct bond, hydrogen,
 (C₁-C₁₀)alkyl substituted with 0-3 R⁷², aryl
 substituted with 0-3 R⁷², cycloalkyl substituted
 with 0-3 R⁷², heterocycloalkyl substituted with
 20 0-3 R⁷², aralkyl substituted with 0-3 R⁷², alkaryl

- substituted with 0-3 R⁷² substituted with 0-3 R⁷²
 and an electron, provided that when one of R⁷⁰ or
 R⁷¹ is an electron, then the other is also an
 electron, and provided that when one of R⁷² or R⁷³
 5 is an electron, then the other is also an
 electron;
 additionally, R⁶⁰ and R⁶¹ may combine to form =C(C₁-
 C₃)alkyl (C₁-C₃)alkyl;
 R⁷² is independently selected at each occurrence from
 10 the group: a direct bond, =O, F, Cl, Br, I, -CF₃,
 -CN, -CO₂R⁷³, -C(=O)R⁷³, -C(=O)N(R⁷³)₂, -CHO,
 -CH₂OR⁷³, -OC(=O)R⁷³, -OC(=O)OR^{73a}, -OR⁷³,
 -OC(=O)N(R⁷³)₂, -NR⁷³C(=O)R⁷³, -NR⁷⁴C(=O)OR^{73a},
 -NR⁷³C(=O)N(R⁷³)₂, -NR⁷⁴SO₂N(R⁷³)₂, -NR⁷⁴SO₂R^{73a},
 15 -SO₃H, -SO₂R^{73a}, -SR⁷³, -S(=O)R^{73a}, -SO₂N(R⁷³)₂,
 -N(R⁷³)₂, -NHC(=NH)NHR⁷³, -C(=NH)NHR⁷³, =NOR⁷³,
 NO₂, -C(=O)NHR⁷³, -C(=O)NHN(R⁷³)R^{73a}, -OCH₂CO₂H,
 2-(1-morpholino)ethoxy,
 (C₁-C₅)alkyl, (C₂-C₄)alkenyl, (C₃-C₆)cycloalkyl, (C₃-
 20 C₆)cycloalkylmethyl, (C₂-C₆)alkoxyalkyl,
 aryl substituted with 0-2 R⁷³,
 a 5-10-membered heterocyclic ring system containing 1-
 4 heteroatoms independently selected from N, S,
 and O;
 25 R⁷³, R^{73a}, and R⁷⁴ are independently selected at each
 occurrence from the group: a direct bond, (C₁-
 C₆)alkyl, phenyl, benzyl, (C₁-C₆)alkoxy, halide,
 nitro, cyano, and trifluoromethyl; and
 Pg is a thiol protecting group capable of being
 30 displaced upon reaction with a radionuclide.

26. The method of Claim 24 wherein C_h is selected from
 the group:
 diethylenetriamine- pentaacetic acid (DTPA);
 35 ethylenediamine-tetraacetic acid (EDTA); 1,4,7,10-
 tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid
 (DOTA);
 1,4,7,10-tetraaza-cyclododecane-N,N',N''-triacetic acid;

hydroxybenzyl-ethylene-diamine diacetic acid;
 N,N'-bis(pyridoxyl- 5-phosphate)ethylene diamine;
 N,N'-diacetate, 3,6,9-triaza-12- oxa-3,6,9-
 tricarboxymethylene-10-carboxy-13-phenyl-tridecanoic acid;
 5 1,4,7-triazacyclononane-N,N',N''-triacetic acid;
 1,4,8,11- tetraazacyclo-tetradecane-N,N'N'',N'''-
 tetraacetic acid;
 2,3-bis(S- benzoyl)mercaptoacetamido-propanoic acid.

10 27. The method of Claim 24 wherein M_t is indium-111 or
 gallium-68.

28. A method for Claim 4 wherein the neurological disease
 is Alzheimer's disease.

15

29. A method for diagnosing a neurological disease
 involving APP processing and/or the production of beta-
 amyloid production within a mammalian body comprising:

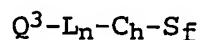
(a) administering a diagnostically effective amount of
 20 an ultrasound contrast agent composition inhibitor of APP
 processing and/or the production of beta-amyloid
 production; and

(b) imaging the area of the patient wherein the
 disease is located.

25

30. A method for diagnosing a neurological disease
 according to Claim 29, wherein the an ultrasound contrast
 agent composition is of the formula

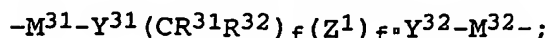
30



wherein

Q^3 is an inhibitor of APP processing and/or the production
 of beta-amyloid production;

35 L_n is a linking group of the formula



wherein

M^{31} is $-(CH_2)_g Z^{31}]_{g'}-(CR^{31}R^{32})_{g''}-$;

M^{32} is $-(CR^{31}R^{32})_{g''}-[Z^{31}(CH_2)_g]_{g'}-$;

g is independently 0-10;

5 g' is independently 0-1;

g'' is independently 0-10;

f is independently 0-10;

f' is independently 0-10;

f'' is independently 0-1;

10 Y^{31} and Y^{32} , at each occurrence, are independently selected from: a direct bond, $-O-$, $-NR^{32}-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)O-$, $-C(=O)NH-$, $-C(=NR^{32})-$, $-S-$, $-SO-$, $-SO_2-$, $-SO_3-$, $-NHC(=O)-$, $-(NH)_2C(=O)-$, $-(NH)_2C=S-$;

15 Z^{31} is independently selected at each occurrence from a (C_6-C_{14}) saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{33} ; and a heterocyclic ring system, optionally substituted with 0-4 R^{33} ;

20 R^{31} and R^{32} are independently selected at each occurrence from: hydrogen; (C_1-C_{10})alkyl substituted with 0-5 R^{33} ; alkaryl wherein the aryl is substituted with 0-5 R^{33} ;

R^{33} is independently selected at each occurrence from the group: hydrogen, $-OH$, $-NHR^{34}$, $-C(=O)R^{34}$, $-OC(=O)R^{34}$, $-OC(=O)OR^{34}$, $-C(=O)OR^{34}$, $-C(=O)NR^{34}$, $-CN$, $-SR^{34}$, $-SOR^{34}$, $-SO_2R^{34}$, $-NHC(=O)R^{34}$, $-NHC(=O)NHR^{34}$, or $-NHC(=S)NHR^{34}$;

25 and

R^{34} is independently selected at each occurrence from the group: hydrogen; (C_1-C_6)alkyl; benzyl, and phenyl;

S_f is a surfactant which is a lipid or a compound of the

30

formula: $A^g E^1 A^{10}$;

A^g is selected from the group: OH and OR^{27} ;

A^{10} is OR^{27} ;

R^{27} is $C(=O)C_{1-20}$ alkyl;

35 E^1 is C_{1-10} alkylene substituted with 1-3 R^{28} ;

R^{28} is independently selected at each occurrence from the group: R^{30} , $-PO_3H-R^{30}$, $=O$, $-CO_2R^{29}$, $-C(=O)R^{29}$,

-C(=O)N(R²⁹)₂, -CH₂OR²⁹, -OR²⁹, -N(R²⁹)₂, C₁-C₅ alkyl, and C₂-C₄ alkenyl;

R²⁹ is independently selected at each occurrence from the group: R³⁰, H, C₁-C₆ alkyl, phenyl, benzyl, and

5 trifluoromethyl;

R³⁰ is a bond to L_n;

and a pharmaceutically acceptable salt thereof.

10 31. The method according to Claim 30, wherein the ultrasound contrast agent composition further comprises: 1,2-dipalmitoyl-sn-glycero-3-phosphotidic acid, 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine, and N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-
15 dipalmitoyl-sn-glycero-3-phosphatidylethanolamine.

32. The method according to Claim 30, wherein the ultrasound contrast agent composition further comprises an echogenic gas.

20

33. The method according to Claim 32, wherein the echogenic gas is C₂₋₅ perfluorocarbon.

25

34. A method of in vivo diagnostic imaging comprising administering to a subject a diagnostically effective amount of a radiolabeled inhibitor of beta-amyloid production.

30

35. A method of Claim 34 wherein said method is used in the diagnosis of a neurological disease which involves APP processing or elevated levels of beta-amyloid, or both.

35 36. A method of Claim 34 wherein said method is used in the diagnosis of Alzheimer's disease.

37. A method of Claim 34 wherein the radiolabeled inhibitor is suitable for imaging of the brain of the subject.
- 5 38. A method of Claim 34 wherein the radiolabeled inhibitor is radiolabeled with one or more radioisotope selected from ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{123}I , ^{125}I , or ^{131}I .
- 10 39. A method of Claim 34 wherein the inhibitor of beta-amyloid production is a compound selected from the group consisting of 1) any compound Claimed in a reference listed in Table 2, 2) any compound within the scope of compounds disclosed in a reference listed in Table 2, and
- 15 3) any compound which inhibits beta-amyloid production and binds competitively with any of the foregoing compounds in any of the assays described in the Utility section hereof.
- 20 40. A method of Claim 34 wherein the inhibitor of beta-amyloid production exhibits activity as an inhibitor in the method of screening for inhibitors of beta-amyloid production comprising,
- 25 1) contacting a potential inhibitor of beta-amyloid production and a tagged inhibitor of beta-amyloid production with at least one macromolecule involved in the processing of APP and the production of beta-amyloid peptide, said macromolecule containing a binding site specific for said tagged inhibitor of beta-amyloid production;
- 30 2) separating the tagged inhibitor of beta-amyloid production bound to said macromolecule from the tagged inhibitor of beta-amyloid production free from said macromolecule; and
- 35 3) determining an inhibitory concentration of the potential inhibitor of beta-amyloid production from the concentration of tagged inhibitor of beta-amyloid production bound to said macromolecule.

41. A method of Claim 34 wherein the inhibitor of beta-amyloid production binds to a macromolecule which is capable of being identified by the method of identifying a macromolecule involved in APP processing comprising

- 1) contacting a tagged inhibitor of beta-amyloid production with material suspected to contain a macromolecule involved in APP processing;
- 2) separating a complex comprising a tagged inhibitor of beta-amyloid production and a macromolecule involved in APP processing; and
- 3) identifying the complex.

42. A method of Claim 34 wherein the inhibitor of beta-amyloid production binds to a macromolecule involved in APP processing comprising a macromolecule to which a tagged inhibitor of beta-amyloid production binds to specifically.

43. A method of Claim 34 wherein the inhibitor of beta-amyloid production is selected from an inhibitor of beta-amyloid production comprising a compound which interacts with a binding site on a macromolecule involved in the production of beta-amyloid peptide; wherein said binding site is a specific binding site for a compound of Formula (I-7T) or (I-43T) wherein m is about 2.

44. A method of Claim 34 wherein the radiolabeled inhibitor of beta-amyloid production is a radiolabeled tagged inhibitor of beta-amyloid production comprising a tagged compound which interacts with a binding site on a macromolecule involved in the production of beta-amyloid peptide; wherein said binding site is a specific binding site for a compound of Formula (I-7T) or (I-43T) wherein m is about 2.

45. A method of Claim 34 wherein the inhibitor of beta-amyloid production is selected from:

- (1) an inhibitor of presenilin-1;
- 5 (2) an inhibitor of presenilin-2;
- (3) an inhibitor of β secretase;
- (4) an inhibitor of α secretase;
- (5) an inhibitor of γ secretase; or
- (6) an inhibitor of BACE/memapsin 2.

10

46. A pharmaceutical composition suitable for in vivo diagnostic imaging comprising a radiolabeled inhibitor of beta-amyloid production.

15 47. A pharmaceutical composition of Claim 46 wherein the composition is used in the diagnosis of a neurological disease which involves APP processing or elevated levels of beta-amyloid, or both.

20 48. A pharmaceutical composition of Claim 46 wherein the composition is used in the diagnosis of Alzheimer's disease.

49. A pharmaceutical composition of Claim 46 wherein the
25 radiolabeled inhibitor is suitable for imaging of the brain of the subject.

50. A pharmaceutical composition of Claim 46 wherein the radiolabeled inhibitor is radiolabeled with one or more
30 radioisotope selected from ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{123}I , ^{125}I , or ^{131}I .

51. A pharmaceutical composition of Claim 46 wherein the inhibitor of beta-amyloid production is a compound selected
35 from the group consisting of 1) any compound Claimed in a reference listed in Table 2, 2) any compound within the scope of compounds disclosed in a reference listed in Table 2, and 3) any compound which inhibits beta-amyloid

production and binds competitively with any of the foregoing compounds in any of the assays described in the Utility section hereof.

5 52. A pharmaceutical composition of Claim 46 wherein the inhibitor of beta-amyloid production is selected from:

- (1) an inhibitor of presenilin-1;
- (2) an inhibitor of presenilin-2;
- (3) an inhibitor of β secretase;
- 10 (4) an inhibitor of α secretase;
- (5) an inhibitor of γ secretase; or
- (6) an inhibitor of BACE/memapsin 2.

FIG. 1

Binding

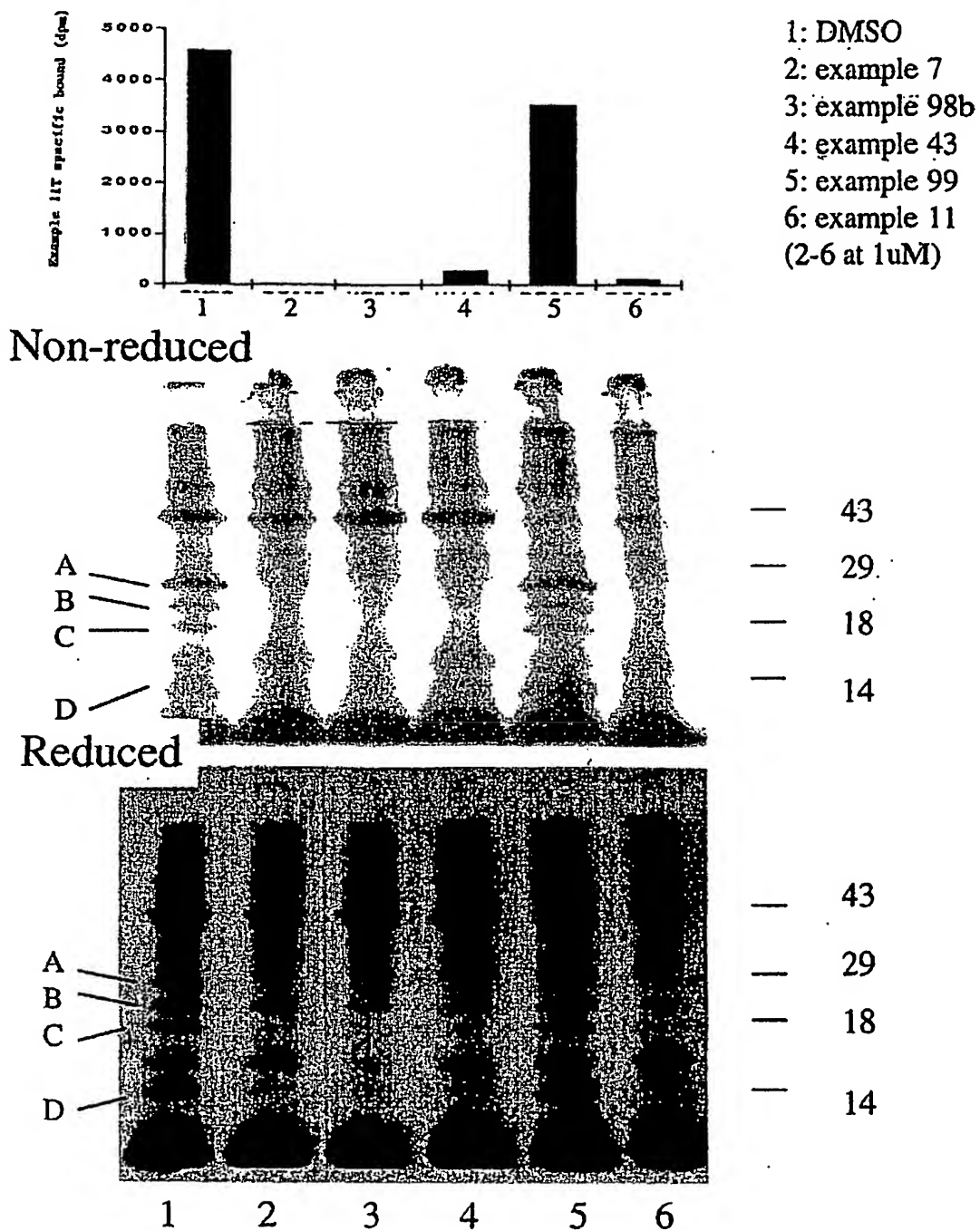


FIG. 2

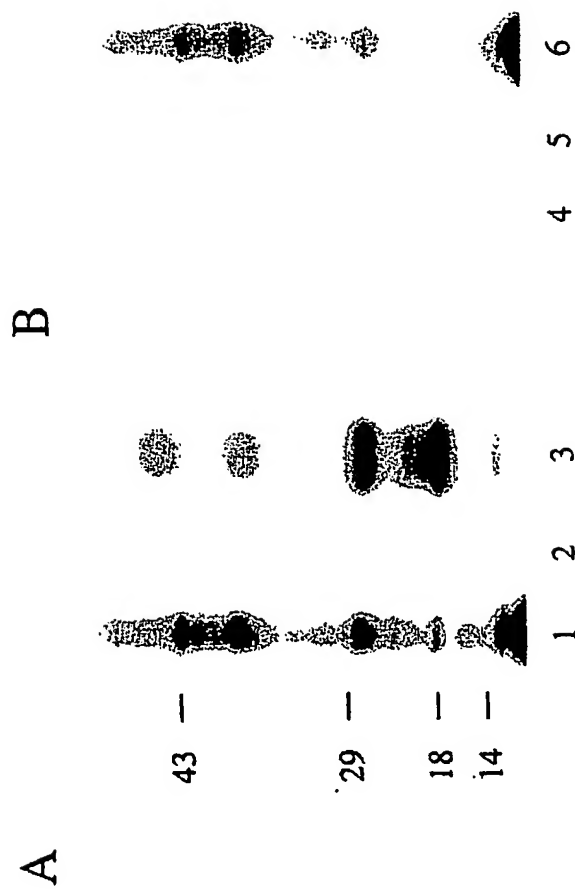
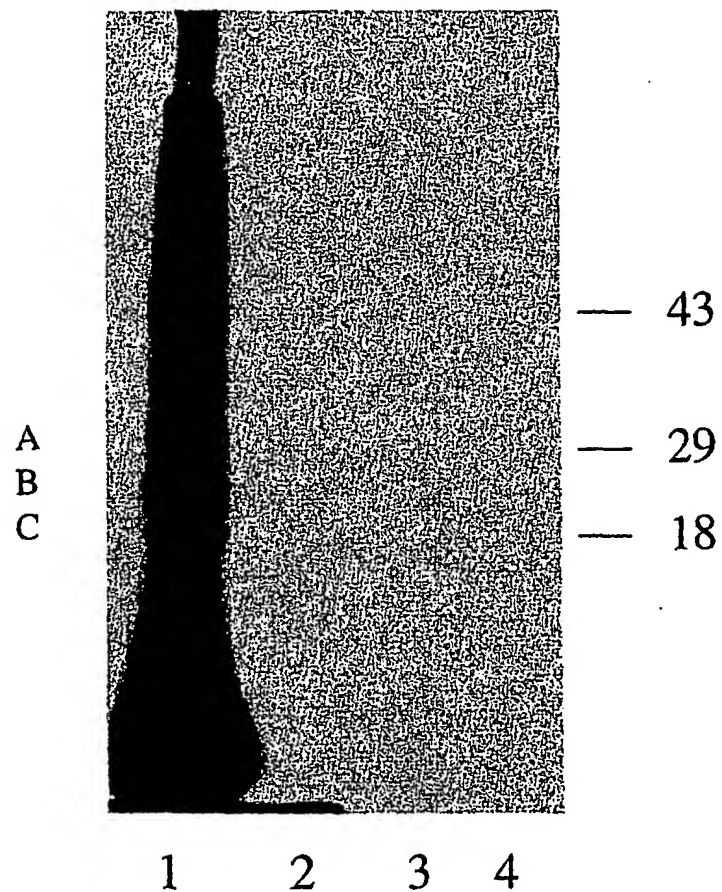


FIG. 3



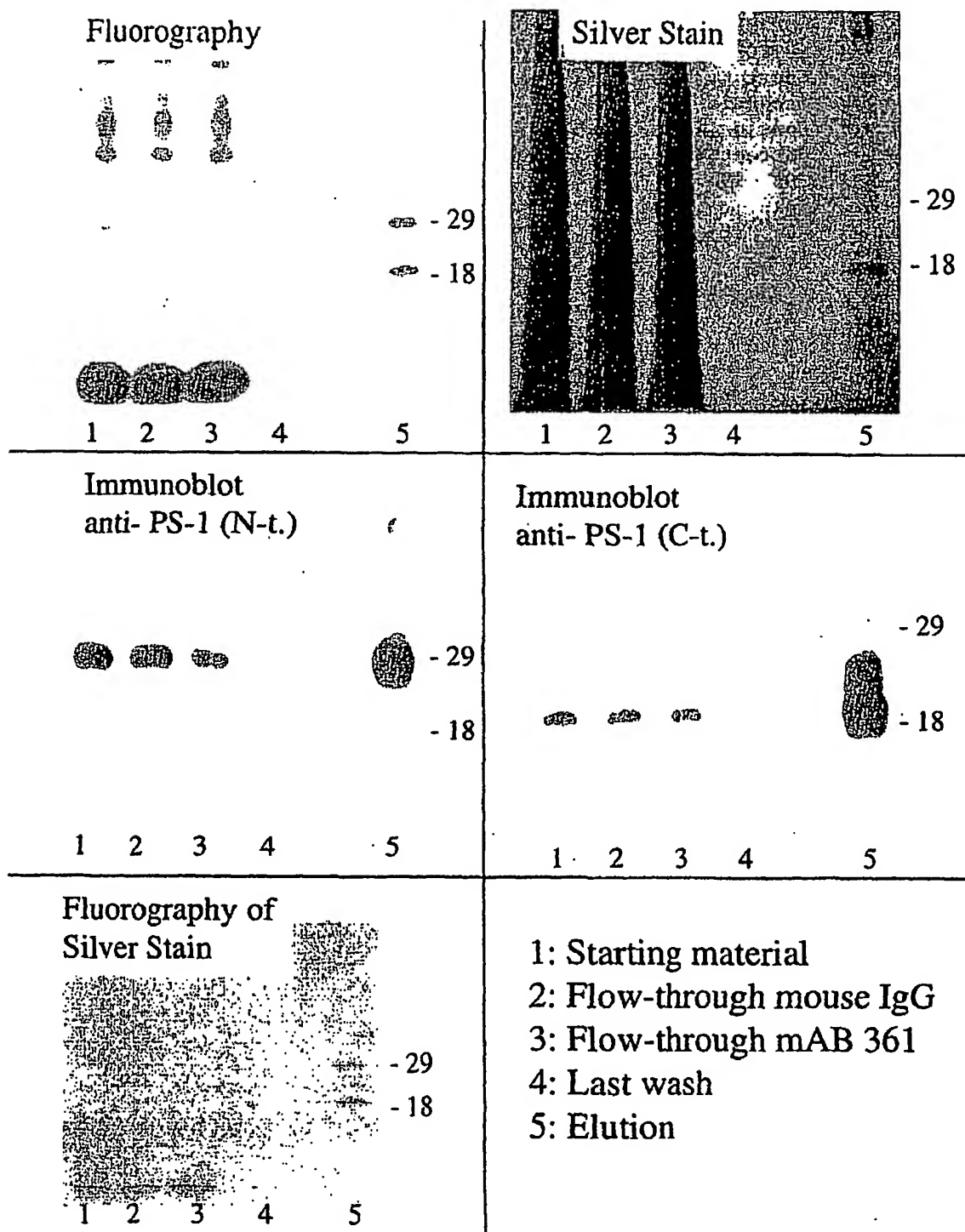
1: Starting material

2: I.P. PS-2 N-t.

3: I.P. PS-2 C-t. loop

4: I.P. normal rabbit IgG

FIG. 4



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(74) Agent: LARSEN, Scott, K.; Bristol-Myers Squibb
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Princeton, NJ 08543-4000 (US).

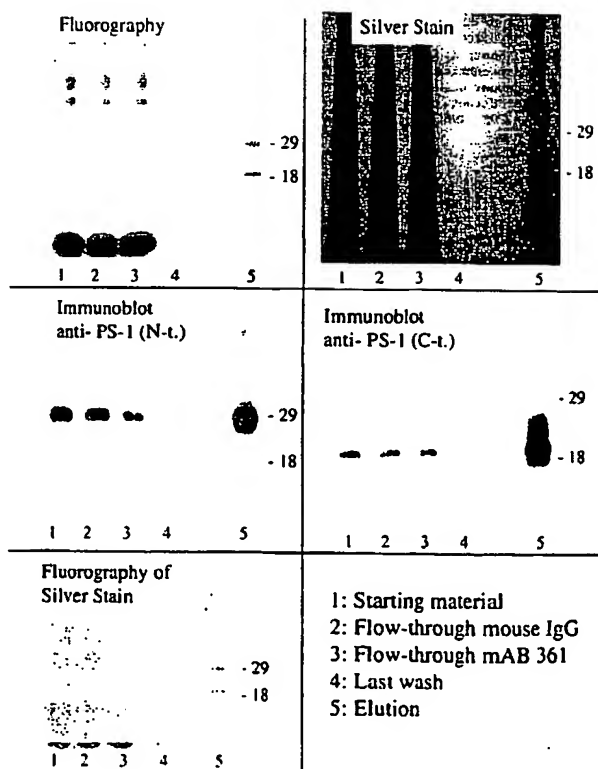
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DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
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TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.(71) Applicant: BRISTOL-MYERS SQUIBB PHARMA
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[Continued on next page]

(54) Title: USE OF SMALL MOLECULE RADIOLIGANDS FOR DIAGNOSTIC IMAGING

(57) Abstract: This invention relates to a method of using radiolabelled and/or radiopharmaceutical small molecule inhibitors of beta-amyloid peptide production for the diagnosis of neurological and other disorders involving APP processing and beta-amyloid production. Furthermore, radiolabelled small molecule inhibitors identified by the methods of the present invention would be useful in the diagnosis of neurological disorders, such as Alzheimer's disease, which involve elevated levels of A β peptides.



IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SEIFFERT D ET AL: "Presenilin-1 and -2 are molecular targets for gamma-secretase inhibitors." JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 NOV 3) 275 (44) 34086-91. , XP002198911 abstract; figure 4; table 1	1
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-52 relate to an extremely large number of possible compounds. In fact, the claims contain so many options or variables, that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely the examples and closely related compounds.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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